

INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI

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GIPNLK—4/JDIARI/60—16-3-61—5,000

University of California Publications in
ZOOLOGY

VOLUME XXXI

EDITORS

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UNIVERSITY OF CALIFORNIA PRESS
BERKELEY, CALIFORNIA
1929

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THE RELATIONS AND NATURE OF THE CUTANEOUS
VESSELS IN SELACHIAN FISHES

BY

J FRANK DANIEL AND EDITH STOKER

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 1, pp. 1-6, 4 figures in text

Issued June 30, 1927

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

THE RELATIONS AND NATURE OF THE CUTANEOUS VESSELS IN SELACHIAN FISHES

BY

J. FRANK DANIEL AND EDITH STOKER

INTRODUCTION

The system of vessels in the integument of elasmobranch fishes consists of unpaired and paired longitudinal trunks and their tributaries. The unpaired vessels lie in the middorsal and midventral lines and the paired lateral vessels accompany the series of lateral line organs. In certain types of sharks there is an accessory lateral vessel, the inferior lateral cutaneous of Sappey (1843), running between pectoral and pelvic areas parallel to the lateral vessel (see Daniel, 1922).

The above trunks and their tributaries were accurately studied in this laboratory by Dr. Helen Hopkins but, owing to uncertainty as to the deeper connections of some of these vessels, her paper was not published. Moreover, while the evidence for considering these vessels as veins was strong, we were at the time unable to demonstrate their true nature. The present paper deals with the connections and nature of these vessels.

RELATION OF THE CUTANEOUS TRUNKS TO DEEPER VESSELS

RELATIONS OF THE DORSAL CUTANEOUS SYSTEM

The dorsal cutaneous vessel forms loops around the dorsal fin. At the posterior part of the loop back of the second dorsal fin there is a relatively large sinus into which the posterior segment of the dorsal cutaneous (*d.c.²*, fig. 2) empties. Passing forward from the sinus and forming the arms of the loop are the *venae circulares* (*c.v.²*) of Mayer (1888). These vessels meet in front of the fin. From the sinus also are two relatively large vessels which pass forward and obliquely downward. These vessels unite to form the *vena profunda* (*pr.²*) of

Mayer. In *Squalus sucklui* and *Mustelus henlei* after this deep branch has passed the spinal column it joins the caudal vein (*c.d.*).

The loop around the first dorsal fin in *Mustelus henlei* (fig. 1) is much like that described by Parker (1886) for *Mustelus antarcticus*. In fact, the composition of the loop and the arrangement of the vessels associated with it are essentially alike in both of the dorsal fins. The

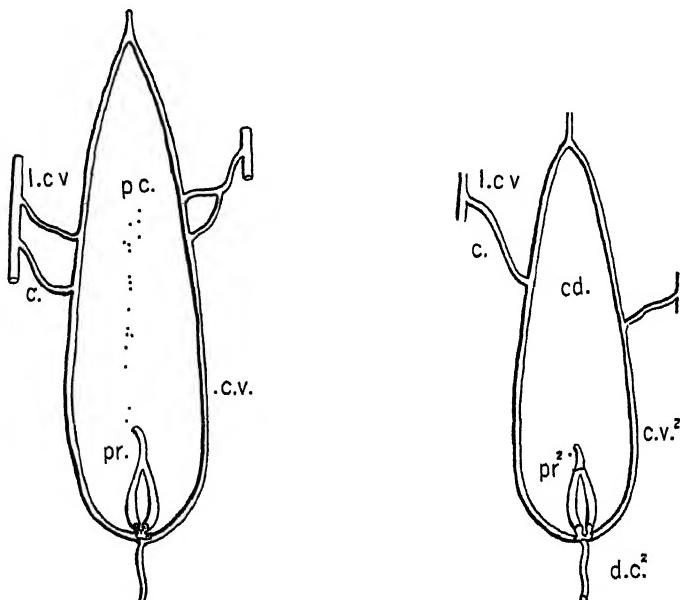


Fig. 1. Cutaneous loop around first dorsal fin, *Mustelus henlei*, dorsal view. *c.*, connection between vena circularis and lateral cutaneous vessels; *c.v.*, vena circularis of first dorsal fin; *l.c.v.*, lateral cutaneous vessel; *p.c.*, postcardinal vein; *pr.*, vena profunda of first dorsal fin.

Fig. 2. Cutaneous loop around second dorsal fin, *Mustelus henlei*, dorsal view. *c.*, connection between vena circularis and lateral cutaneous vessel; *c.d.*, caudal vein; *c.v.²*, vena circularis of second dorsal fin; *d.c.²*, dorsal cutaneous vessel; *l.c.v.*, lateral cutaneous vessel; *pr.²*, vena profunda of second dorsal fin.

terminal relations of the vena profunda (*pr.*) of the first dorsal fin differ considerably in different types of sharks. The most usual condition in *Squalus sucklui* is for this deep vessel to pass down on the left side of the spinal column. If it passes to the left of the column the vessel strikes the mesonephros at the end of the left postcardinal vein. Instead of emptying into this vein, however, it crosses over to the opposite side as a cross-trunk and enters the right, longer, postcardinal vein. In *Mustelus henlei* the vena profunda may terminate in essentially the same position but it joins the short postcardinal (*p.c.*, fig. 1)

and does not pass over to the opposite side by a cross-trunk. When it passes to the right of the column in *Mustelus henlei* it breaks up in a leash on the mesonephros; in this case it may join the renal portal vein.

The most anterior segment of the dorsal cutaneous vessel enlarges considerably as it runs forward from the first dorsal fin. Just before reaching the endolymphatic ducts it divides into right and left divisions as is shown in figure 3. In *Squalus* each of these divisions (*o.v.*), after receiving one or several branches from the skin above the orbit, perforates the supraorbital crest and empties into the postorbital sinus in the orbit (*or.*). This sinus is a part of the anterior cardinal system. In *Mustelus henlei* the cutaneous twigs above the orbit and above the branchial area form a common trunk which enters the anterior cardinal directly.

RELATIONS OF THE LATERAL AND ACCESSORY LATERAL CUTANEOUS VESSELS

The lateral cutaneous vessel (*l.c.v.*, figs. 1, 2), in addition to being connected to both dorsal cutaneous loops by strong communicating branches (*c.*), is also connected segmentally to the accessory lateral vessel. Anteriorly the lateral cutaneous and accessory lateral cutaneous vessels join the subscapular sinus. As was previously shown (Daniel, 1918, p. 483; 1922, p. 226) the content of these vessels after entering the subscapular sinus is emptied either through the subclavian vein or through the postcardinal sinus. In *Heptanchus maculatus* it continues down the well formed subscapular vein to the subclavian vein and thence to the heart. In *Squalus sucklii* the subscapular sinus may come in contact and make connection with the postcardinal sinus. The content from the lateral cutaneous in this case has two courses open to it; it may enter either the postcardinal sinus or the subclavian vein. In *Mustelus henlei*, as in *M. antarcticus* and in *Scyllium canicula* (O'Donoghue, 1914), the lateral cutaneous enters the subscapular sinus from which its content is poured into the postcardinal sinus.

RELATION OF THE VENTRAL CUTANEOUS VESSEL

The relations of the ventral cutaneous in the cloacal region are more complex. The postcloacal segment of the vessel (*p.c.*, figs. 4) is shaped like the letter *Y*, the arms of which run in the integument on the dorsal side of the pelvic fins. These arms terminate in a sinus (*s.*) where the fin joins the body. This sinus receives a second strong cutaneous branch (*pl.*) from the precloacal segment of the ventral cutaneous which, because of its relation to the pelvic girdle, we have designated as the pelvic vessel. The sinus, after receiving these

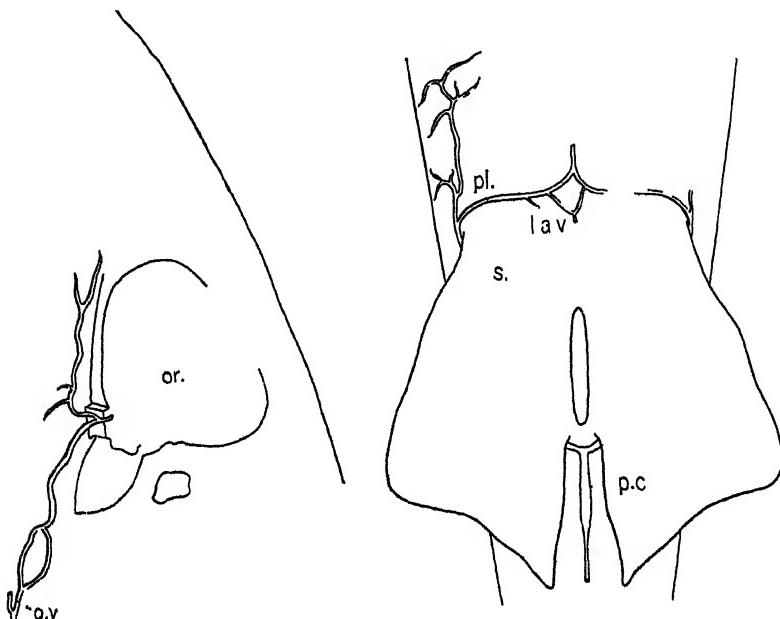


Fig. 3. Cutaneous vessel entering orbital sinus, *Squalus sucklii*, dorsal view. *or.*, orbit; *ov.*, orbital vessel.

Fig. 4. Cutaneous vessels in pelvic area, *Squalus sucklii*, ventral view. *l.a.v.*, lateral abdominal vein; *p.c.*, posterior segment of ventral cutaneous vessel; *pl.*, pelvic vessel; *s.*, sinus.

terminal vessels of the postcloacal and precloacal segments of the ventral cutaneous, then makes its deeper connection with the lateral abdominal vein (*l.a.v.*).

In the region of the pectoral girdle the postcoracoid and precoracoid segments of the ventral cutaneous join the coracoid vessel as was previously described (Daniel, 1918). It is through the coracoid vein that they make their deeper connection with the lateral abdominal system.

THE NATURE OF THE CUTANEOUS VESSELS

HISTORICAL

Monro (1785), who early worked on selachian fishes, considered the cutaneous vessels to be lymphatic in nature. Later, a number of investigators added evidence which indicated that they were haemal in nature. Parker (*op. cit.*) observed that the vessels may contain blood. Mayer (*op. cit.*) reported valves in the vessels and further stated that he had observed blood circulating in the vessels of a semi-transparent embryo. But similar cutaneous vessels in types above the elasmobranch fishes have almost universally been regarded as lymphatic.

EXPERIMENTAL

In order to convince ourselves of the nature of these vessels in Selachia we have made use of the experimental method. Through the kindness of Dr. B. W. Evermann and Mr. Seale we were provided with living specimens of *Squalus sucklui* and *Mustelus henlei* and were given every facility for a study of the problem at the Steinhart Aquarium in San Francisco.

The method used was as follows: The animal was turned on its side and strapped in place in a trough. About five cubic centimeters of ether was added to the sea water in the trough, and the water stirred so that as much of the ether would mix with the water as possible. Generally the animal was under the anaesthetic within fifteen minutes, after which water was allowed to flow slowly into the trough. An incision was then made through the lateral cutaneous vessel in the region half-way between the anterior margin of the dorsal fin and the pectoral fin, and a glass canula about one-fourth millimeter in diameter was inserted into the distal end of the vessel. The vessel was then ligatured over the canula.

The results of this experiment were easily followed. There was practically no flow from the proximal end of the incised vessel, but from the distal end, the one in which the canula had been placed, there was a rapid rush of blood out through the canula. After a few seconds the flow became less rapid, and finally continued as a slow steady flow.

From the above experiment we conclude that the cutaneous system of vessels in these two typical selachians, *Squalus sucklui* and *Mustelus henlei*, on which we have made our experimental study, is haemal in nature. The vessels, therefore, should be described as veins.

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ON COUNCILMANIA DISSIMILIS SP. NOV.,
AN INTESTINAL AMOEBA FROM MAN

BY

CHARLES ATWOOD KOFOID

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 2, pp. 7-16, plates 1 and 2

Issued August 23, 1927

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

ON COUNCILMANIA DISSIMILIS SP. NOV.,
AN INTESTINAL AMOEBA FROM MAN

BY
CHARLES ATWOOD KOFOID

There is a species of parasitic intestinal amoeba found in human faeces, which at present masquerades under the cover of *Endamoeba dysenteriae*. It resembles this well-known pathogenic species very closely in many structural characteristics. It is, indeed, included in some of the published figures of cysts of *E. dysenteriae*, as in those of Hartmann and Belar (1921). It may be responsible for the belief that *E. dysenteriae* sometimes goes to the eight-nucleate stage in the cyst (for discussion see Dobell, 1919, p. 49) and that this species sometimes exhibits the appearance of budding. It undoubtedly is being reported for treatment, on the basis of the findings on faecal examinations, as *E. dysenteriae*. To what extent its presence in man and its confusion at present with *E. dysenteriae* (large race) complicate the clinical picture of amoebiasis and of amoebic therapy, can be determined only by prolonged study. It is important that this species be clearly recognized in stool examination and ruled out of possible confusion with larger races of *E. dysenteriae*, especially by critical clinical and therapeutic investigations and comparisons of cases of pure infections by the two species singly. The characters of this species of Councilmania are as follows:

Councilmania dissimilis sp. nov.

Plates 1 and 2

The outstanding features of this species are: The heavy lateral blob of peripheral chromatin on the nuclear membranes and the predominantly dispersed karyosomes in the matured cysts, the occurrence of eight-nucleate cysts, and the free budding of cysts in the faeces.

The nucleus is typically spherical, but seems subject to slight deformations, somewhat more than in *E. dysenteriae* and not to the extent seen in *E. gingivalis*. The nuclear membrane is faint, especially in cysts and in the chromatin-free parts. Its chromatin occurs in

two regions, the peripheral, applied to the nuclear membrane, and the karyosomal, or central, which usually is considerably dispersed.

The peripheral chromatin has a characteristic distribution. Instead of being distributed over the inner surface of the nuclear membrane in a thin layer, or scattered in small masses of subuniform or somewhat varying size as it is in *E. dysenteriae*, we find it in *C. dissimilis* massed mainly, or even almost wholly, in one large lateral blob. This single mass is crescentic in outline, is spread over almost one-half the circumference of an optical section of a nucleus when the blob is in the plane of the section. It may appear shorter than this, but in such cases will usually be found to be foreshortened by the obliquity of its position with reference to the focal plane. Its greatest thickness ranges from 0.14 to 0.30 of the nuclear diameter and its width somewhat greater. It stains intensely black because of its mass. Its inner face may show evidence of indentation or subdivision. When it lies on the upper or lower surface of the nucleus it may be overlooked.

In the relatively infrequent binucleate stage the lateral blobs are reduced or may even be absent. In some nuclei there may be small accumulations of peripheral chromatin elsewhere than in the blob, which appear in optical section as thin blobs or scattered granules applied to the nuclear membrane. In some instances in motile forms in culture the blob is considerably reduced in thickness and the peripheral extension of the chromatin increased. The most striking feature of this species is the structure of the nucleus of the mature cyst with the heavy lateral blob and faint nuclear membrane elsewhere on the nuclear surface. In the binucleate stage, because of its close juxtaposition to the preceding and following mitosis, the lateral blob is less in evidence. Its material is transferred to the polar masses of the spindle during the prophases and a very slender intradeutome is formed between them.

The central karyosome (pl. 1, figs. 4-8) is composed of a number of discrete granules which in the nuclei of the matured cyst are often rather widely dispersed. We find no evidence of a clear halo around the karyosome as in *E. dysenteriae* (for figures see Kofoid and Swezy, 1924, p. 17, figs. 32-43), or of the typical spheroidal state of the karyosome so characteristic of *E. dysenteriae*. The closest approach to this spheroidal form is shown in plate 1, figure 2. This dispersal is strikingly similar in pattern to that described in *Councilmania lafleuri* by Kofoid and Swezy (1921) and to that found by Kessel (1924) in *C. muris* and *C. decumani* from rats and mice.

The number of nuclei in the cyst depends on the age of the cyst. In some stools mononucleate cysts with large nuclei are abundant, many in the prophases of mitosis. In others four-nucleate cysts predominate with few mononucleates and binucleates. Less frequently, and generally in stools with mature, four-nucleated cysts, there occur in some stools a few with eight nuclei. This number, and that of four-nucleates also, are reduced by budding and, in some instances, by the successive escape of amoebulae. Four-nucleate cysts are frequently seen with prophases of mitosis in their nuclei.

We have never seen this increase in the number of nuclei from four to eight in the cysts in *Endamoeba dysenteriae*. The cases, stools, and smears in which the eight-nucleate cysts occur in *C. dissimilis* are, however, so rare that this feature is available only infrequently for diagnostic purposes in stool examination. It is, however, of classificatory value as a mark of a distinct species.

The number of chromosomes in this species is *eight*, whereas in *Endamoeba dysenteriae* the number is six. The chromosomes can be counted in the prophase as split, meridional, faintly stained structures on the nuclear membrane and at the late metaphase as bilobed or divided masses of equal or unequal sizes. The material of the lateral blob forms around the polar centrosomes and perhaps contributes to the faint intradesmose joining them.

The glycogen vacuole is characteristically excentric in location, best seen at its maximum development in mononucleate cysts, often contains throughout its entire area in stained cysts a fine reticulum with stained nodal granules, and may fill nearly one-half of the cyst. It is asymmetrically lenticular in contour and crowds close to the periphery. It may be broken up into smaller areas of equal or varying size with somewhat diffuse margins, often larger in the periphery of the cyst. The lateral position of this vacuole is correlated with the crowding of the single nucleus to one side.

The chromatoidal substance typically stains a dense homogeneous black in iron haematoxylin, but in this species exhibits considerably more variability than in *E. dysenteriae*. In addition to the homogeneous condition we find also black-bordered chromatoidal bodies with paler centers, and washed-out, uniformly pale bodies without borders. Likewise there is rather more than the usual evidence of halos (or shrinkage?) about these bodies.

The shape and size of these chromatoidal bodies vary greatly according to the phase of their formation. In the early period of

their history they appear near the surface of the large glycogen vacuole and in their last stages they appear as slender rods in halos of solution. In the earliest phases they are oval or asymmetrically ellipsoidal in outline, not over 2 to 2.5 times as long as wide, rounded but more or less asymmetrical at the two ends and often flattened against the glycogen mass to a thickness half their width. There may be over forty such bodies crowded about the surface of the glycogen mass when the glycogen mass is large. The number present in later stages decreases rapidly until in the four- to eight-nucleate stage there may be only one to four present and in the old cyst none at all. When the glycogen and the chromatoidal substance are small in amount, and also as the cysts with much chromatoidal substance age, there is often only a single large chromatoidal body, or in any event only a small number. The largest may be as long as the diameter of the cyst and 0.2 of the length in width, or short and stout, 0.5 as long as wide, or even nearly square. In favorable views and probably in all cases the two ends are asymmetrical and singly or doubly obliquely faceted with rounded angles. The squarish and rectangular types are usually flattened, the longer types more rounded. Whatever the position, careful focusing often reveals this terminal asymmetry and angularity. Their last remnants are slender rods or irregular flakes.

The process of budding of the cysts occurs, as in *Councilmania lafleuri*, freely in the stool. Budding cysts are found in fresh smears not under the least external pressure, in stained smears, and in cysts formed in culture tubes, and the process of the escape of the amoebulae has been watched under the microscope. The pore through which the budding amoebulae escape is 0.35 to 0.50 the diameter of the nucleus of the four-nucleate cyst. The area of the cytoplasm leading toward the pore stains a little more deeply as though condensed, as may also that of the amoeba outside of the cyst wall. This darker staining is not, as a rule, as marked as it is in *C. lafleuri*. Budding has been observed in cysts with one, four, and eight nuclei. The chromophile ridges or strands in the peripheral cytoplasm, which are so prominent in *C. lafleuri*, are only diffusely and faintly developed in *C. dissimilis*.

The cysts are rather uniformly close to the spherical form, whereas in *C. lafleuri* they tend somewhat more toward the broadly ellipsoidal contour. The range and average diameter noted of ten cysts and the most extreme departure from the spherical in five different cases of *C. dissimilis* are as follows. The diameter used when the cyst was not exactly spherical was the longer one.

Stool no.	Range	Average	Diameters of extremes
29056	11.6 to 14.9	13.1	11.6 by 13.3
52260	11.6 to 14.9	13.4	14.9 by 19.2
63774	11.6 to 12.9	12.9	13.3 by 16.6
63885	13.3 to 16.6	14.4	11.6 by 16.6
71341	11.6 to 16.6	14.3	11.6 by 14.9
		13.6	

Councilmania dissimilis is less frequent in man than either *C. lafleuri* or *C. tenuis* ($=$ *Endamoeba tenuis* Kuenen and Swellengrebel, 1917). In 7,746 examinations of about 2,587 persons in my laboratory between January 1 and May 31, 1927, there were eleven cases of infection, or about 0.4 per cent. In all, we have seen probably more than 100 cases. The cysts are often very abundant in the stool; not infrequently as many as five cysts may be seen in a single oil immersion field, indicating unusually heavy infections. As in the cases of other intestinal infections, pure infections by this species alone are rare. Infection usually occurs with one or more of the commoner species of intestinal Protozoa. It persists in varying frequency during any period of examination, and may decrease markedly or even disappear for a time and then return in the stools.

The grounds for the specific separation of this amoeba from *E. dysenteriae*, with which it is at present confused, are primarily the eight chromosomes as over against the six in that species, the very distinctive large blob of chromatin, typically dispersed karyosome in the matured cyst, that is, on attaining the four- or eight-nucleate stage, the occurrence of eight nuclei in the cyst, and the frequency of budding in the stool. Clinical and cultural grounds will be published elsewhere.

The grounds for placing it in the genus *Councilmania* are primarily the predominantly dispersed karyosome, a feature of nuclear structure in the resting nucleus of the matured cyst which characterizes all species of this genus. The slight tendency to asymmetry or off-the-sphere shape of the cyst, the frequent budding process in the stool, and the slightly developed chromophile condition associated with budding, are also more or less developed in the other species of this genus. In the genus *Endamoeba* ($=$ *Entamoeba*) the karyosome is typically solid and spherical. Neither chromophile ridges nor budding in the fresh stool has been seen by us in what we regard as *E. coli* or in *E. dysenteriae*. This process must of course occur in those species also, in some form, whenever and wherever the

contents of the cyst escape; but this has not been seen by us in stools, either fresh or old.

The relation of *C. dissimilis* to the so-called large race of *E. dysenteriae* is at present problematical. It has thus far probably been included in it. In our own material it by no means constitutes the whole of it, though it does form a considerable sector of it, we estimate not less than 25 per cent. Our extensive records and collections of slides have not been reviewed on this point at present writing. It is certainly of sufficient frequency to enter into the problems of microscopical determination of specific infections, clinical observation, symptomatology, and therapy of human amoebiasis.

I am indebted to Miss Inez Smith, M.A., examiner in my laboratory, for the first detection of the distinctive characteristics of *Councilmania dissimilis*; to Miss Olive Swezy, Ph.D., and to Mrs. Dora Henry, M.A., for drawings of this species; and to the Board of Research of the University of California, to Miss Ellen B. Scripps, Mrs. Margaret B. Fowler, Mr. Franklin P. Nutting, the late Regent P. E. Bowles, and to other friends of the University for grants in aid of this research.

SUMMARY

Councilmania dissimilis sp. nov. from man occurs in the motile, encysted, and budding stages in the faeces. In the motile stage it has clear pseudopodia. Cysts occur in the one-, two-, four-, and eight-nucleate phases. Budding of successive amoebulae through the pore reduces this number of nuclei. Cysts have been observed with an average diameter ranging from 11.6 to 17 microns. The nucleus in both motile and encysted stages has a large lateral blob, with little peripheral chromatin elsewhere, and a dispersed, usually central karyosome. There are eight chromosomes. The percentage of infection in 2,587 persons is about 0.4 per cent.

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EXPLANATION OF PLATES

All figures are of *Councilmania dissimilis* sp. nov., from faecal smears fixed in hot Schaudinn's fluid and stained in iron haematoxylin. All drawings were made with camera lucida, $\times 2500$, except where otherwise stated.

PLATE 1

Drawings by Dr. Olve Swezy.

Fig. 1. Mononucleate cyst with very large spheroidal (probably lenticular in the plane of the section) glycogen vacuole, numerous small faintly bordered chromatoidal bodies, nucleus in early prophase, with peripheral chromatin network and heavy lateral blob.

Fig. 2. Mononucleate cyst with two chromatoidals with unsymmetrical ends, three small glycogen vacuoles, and nucleus with lateral blob, eccentric, irregular karyosome and radii.

Fig. 3. Binucleate cyst with disappearing, diffuse, lenticular, eccentric glycogen vacuole; two small flake-like chromatoidals, nuclei in prophase with emerging chromosomes, the left with an intradesmose connected at one end with the lateral blob.

Fig. 4. Four-nucleate cyst with diffuse vacuolation, linear remnants of the chromatoidals, nuclei with heavy lateral blobs and dispersed karyosomes.

Fig. 5. Eight-nucleate cyst with diffuse vacuolation, and one small vacuole, stout, angled, bordered chromatoidals, nuclei with marked lateral blobs and dispersed karyosomes.

Fig. 6. Budding cyst with three nuclei, one in the escaping amoebula and chromophile cytoplasm in pore and bud. Remnant of a chromatoidal in a halo, and several other minute remnants. Nuclei with lateral blobs and somewhat dispersed karyosomes.

Fig. 7. Cyst with seven nuclei with lateral blobs and variously dispersed karyosomes, four somewhat bordered, squarish, or rod-like chromatoidals, and no glycogen vacuole. The cytoplasm is chromophile toward and in a small circular pore on the upper surface. Probably one amoebula has already escaped, reducing the eight nuclei to seven.

Fig. 8. Motile amoeba with retracted clear pseudopodia and nucleus with lateral blob and dispersed karyosome.

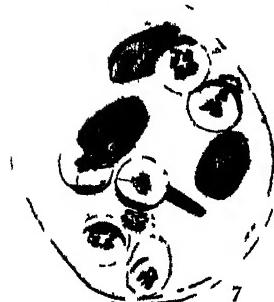
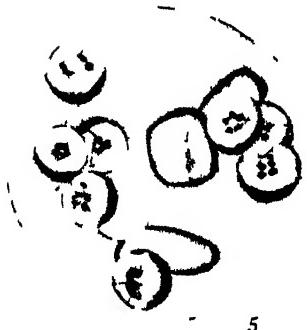


PLATE 2

Drawings by Mrs. Dora Henry.

Fig. 9. Mononucleate cyst with large, lateral, asymmetrically lenticular glycogen vacuole with stained nodal network. Nucleus in early metaphase with eight undivided chromosomes, and slender, linear intradesmose joining two polar masses derived from the lateral blob of peripheral chromatin. Centrosomes, if present at the ends of the intradesmose, are not visible. A single rod-shaped chromatoidal with asymmetrical ends lies below the nucleus.

Fig. 10. Nucleus only, from another mononucleate cyst with more advanced stage of division of the chromosomes. Note differences in size and state of division of the chromosomes. $\times 5000$.

Fig. 11. Mononucleate cyst in more advanced stage of nuclear division than figures 9 and 10. Slender intradesmose joins ends of the polar siderophile masses. Single lateral glycogen vacuole and single chromatoidal rod with squarish ends.

Fig. 12. Binucleate cyst with lateral glycogen vacuole, three chromatoidals, the largest with markedly asymmetrical, subangular ends and deeply stained peripheral layer. Nuclei with lateral blobs. Chromosomes visible in right nucleus.

Fig. 13. Mononucleate cyst with single, flattened, asymmetrically chromatoidal with subangular ends and dark peripheral layer. Large glycogen vacuole present. Nucleus with lateral blob and chromatin in strands on the membrane.

Fig. 14. Four-nucleate cyst with two glycogen vacuoles, two rod-shaped chromatoidals with asymmetrical, subangular ends and peripheral, siderophile layer.

Fig. 15. Eight-nucleate cyst with chromophile cytoplasm emerging from pore.

Fig. 16. Binucleate cyst with amoebula emerging. Compare size of nuclei with those in figure 15. Probably six amoebulae have already escaped.



9



10



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15



16

RELATION OF MOISTURE AND TEMPERATURE
TO THE VIABILITY OF
ENDAMOEBA GINGIVALIS (GROS) IN VITRO

BY

DOROTHY ANN KOCH

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 3, pp. 17-29, 2 figures in text

Issued September 3, 1927

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

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INTRODUCTION AND ACKNOWLEDGMENTS

In an infection as widespread and as common as is that of *Endamoeba gingivalis* of the human mouth, the possible modes of dissemination and the factors influencing its spread are of importance and interest. The distribution of those parasitic Protozoa which have an intermediate host or a resistant or encysted phase may easily be understood. When, however, we encounter, as in the case of this amoeba, a protozoan which has, in so far as has been determined, neither of these two phases in its life-history, it is not so easy to account for its widespread recurrence as a human infection. It would seem, under the circumstances, that resistance to changes in temperature and to desiccation would be the most important factors involved in the survival of these amoebae outside of the human mouth, and in their transference from host to host by indirect routes.

The present investigation establishes the possibility that such a resistance plays an important rôle in the transmission of *Endamoeba gingivalis*. The work was carried on under the direction of Professor Charles A. Kofoid at the University of California, and was made possible by grants to the California Stomatological Research Group from the Carnegie Corporation, from the American Dental Association, from the Associated Radiograph Laboratories of San Francisco, and from the California State Dental Association.

EXPERIMENTAL

METHODS

Previous to the successful and simple method of cultivation of parasitic amoebae and flagellates discovered by Boeck and Drbohlav (1925), the generally accepted criterion for viability of protozoan cysts was their staining reaction to dilute eosin or neutral red solutions (Boeck, 1921a, b; Bercovitz, 1921). Feeding cysts to animals is unsatisfactory, since infection is often, for reasons as yet undetermined, comparatively difficult to establish. Recently Yorke and Adams (1926) have shown that a much more reliable criterion than any of the above is the cultivation of active amoebae from cysts that have been exposed to varying temperatures.

The work here to be described, which was started in September, 1925, is also based on the ability of the amoebae to reproduce in artificial culture, after being subjected to experimental conditions. Since *Endamoeba gingivalis* is readily cultivated, the same technique that would be used in a bacteriological study can be followed here for the determination of temperature relations.

The material for the following experiments was our strains 56 and 60, both newly isolated and both having undergone about six transplants when used. As it was found very important to have a newly isolated culture for this work, it was necessary to use the two strains mentioned. By running these two strains simultaneously for a number of the experimental temperatures, it was found that they did not vary in their susceptibility.

The amoebae were grown on a modification (Howitt, 1926a) of Boeck and Drbohlav's (1925) medium. Maximum growth is obtained forty-eight hours after transplantation into this medium, and cultures of this age were used as stock from which the experimental tubes were seeded just before being subjected to the varying temperatures. In the case of bacteria, after the removal of the inhibiting influence, such as a rise or fall of temperature above or below the optimum, there is a period of lag, during which there is no detected multiplication. This lag is also noted in the routine transplanting of strains into fresh media. No such lag has been noticed with amoebae, although Robertson (1924) has noted it in the infusorian *Enchelys*. Therefore one runs

no risk in using a freshly transplanted culture and conforming to the technique ordinarily accepted for the determination of the thermal death point of bacteria. "Board of Health" type test tubes of uniform thickness and diameter were used throughout. All glassware was thoroughly cleaned and rinsed in 1 per cent hydrochloric acid and, before the medium was inoculated, it was warmed to 37° C. In this way, the organisms were not subjected to any shock due to change in temperature prior to the beginning of the experiment. As soon as inoculated, tubes were subjected to the experimental temperature, timed, and removed immediately to an incubator, at temperature of 37°. Inoculation was made as previously described (Koch, 1926a) and controls at 37° were run at the same time and with the same inoculum as for the experimental tubes.

It is fully realized that, in tubes of the diameter used, it takes a short time for the heat of the surrounding water to penetrate the center of the column of liquid culture medium, and that there is a percentage of error here, due to the fact that all organisms in one tube are not subjected to the heat at exactly the same moment or for the same length of time. But, in order to be absolutely sure of an adequate inoculation of amoebae, it is necessary to transfer about 1 ccm. of the 48-hour culture to the experimental tubes containing a total of about 11 ccm. of liquid culture medium. For this reason it was almost impossible to use a smaller tube without diluting too greatly the fresh medium with the somewhat toxic two-day culture.

Viability of cultures was determined by incubating at 37° and examining at the end of 24 and 48 hours. All 48-hour negative tubes were transplanted to fresh media and reexamined 24 hours later. In this way negatives were checked by subculturing and, in a few cases, slight growth was discovered. Cultures were examined both for growth and for the relative number of amoebae surviving (table 1), the counting being done according to the method of Howitt (1926a). No determination of the thermal death point was considered final until two experiments for the given temperature checked.

Temperatures between 25° and 55° were maintained by a constant temperature electrically controlled water bath accurate to within 0.5 of a degree. For 20° it was necessary to use an improvised water bath kept constant to within one degree, and for temperatures below this an electrically controlled low temperature chamber, adjustable to temperatures ranging from that of the room to 0° C. was used. It was not possible in this work to hold the 20° water bath at constant

TABLE I
THE RELATION OF CHANGES IN TEMPERATURE TO THE VIABILITY OF *Endamoeba gingivalis* *in vitro*

Temperature - Centigrade	Number of amoebae in cover slip preparation														Controls 37°			
	Exposure in minutes							Exposure in hours										
	2	5	10	15	20	25	30	1	2	3	4	5	10	18	24	48	(1)	(2)
45°	b	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	10	72	72
50°	b	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	80	80	80
55°	b	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	130	130	130
45°	b	2(1)	3(1)	1	0	0	0	0	0	0	0	0	0	0	0	205	205	205
40°	b	125(1)	200(1)	15(1)	3(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	130	130	130
40°	c	280(1)	88(1)	1082(1)	640(1)	192(1)	248(1)	280(1)	352(1)	35(2)	40(2)	274(2)	480(2)	44(2)	2(3)	0(2)	180	180
35°	b	300(1)	360(1)	816(1)	176(1)	222(1)	424(1)	136(1)	75(1)	60(2)	65(2)	59(2)	62(2)	64(2)	70(2)	70(2)	216	85
30°	b	138(1)	168(1)	192(1)	182(1)	168(1)	320(1)	110(1)	216(1)	30(2)	36(2)	28(2)	30(2)	26(2)	25(2)	0(2)	120(2)	216
25°	a	288(1)	366(1)	344(1)	384(1)	252(1)	232(1)	248(1)	376(1)	200(2)	186(2)	304(2)	256(2)	144(2)	80(2)	176(2)	8(2)	216
20°	b	48(1)	280(1)	376(1)	56(1)	80(1)	72(1)	120(1)	276(1)	2(1)	54(2)	60(2)	38(2)	29(2)	1(2)	0(2)	384	384
15°	b	48(1)	44(1)	56(1)	88(1)	128(1)	48(1)	8(1)	3(1)	3(1)	198(2)	180(2)	200(2)	165(2)	100(2)	48(2)	0(2)	8
10°	b	8(1)	1(1)	5(1)	2(1)	3(1)	5(1)	1(1)	3(1)	3(1)	96(2)	100(2)	100(2)	100(2)	100(2)	48(2)	0(2)	8
5°	b	108(1)	75(1)	120(1)	258(1)	512(1)	152(1)	144(1)	94(2)	96(2)	100(2)	96(2)	144(2)	432(2)	48(2)	0(2)	162	144
0°	b	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)	0(2)	0(2)	0(2)

α_2 = 24-hour incubation at 37°.

=transplant from "b" after 24 hours.

(1) and (2) refer to controls at right
0 = no growth

temperature for more than one hour. The death point for this temperature must therefore be calculated from the graph shown in figure 1. A recording thermometer was kept in the low temperature chamber at all times, and a graph record kept of the temperature for each experiment.

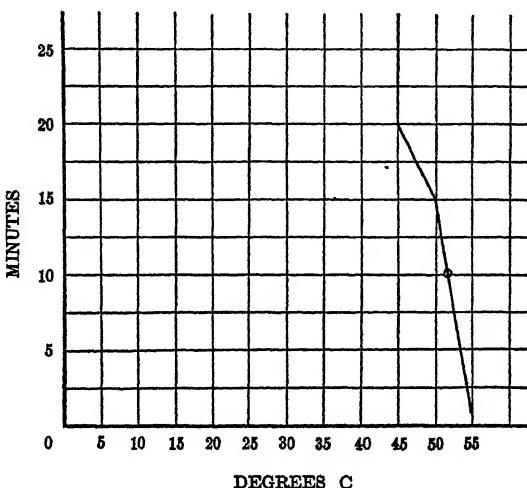


Fig. 1. Graph showing rapid lethal effect of heat above 45° C. upon *Endamoeba gingivalis*.

Magoon (1926), in his studies on bacterial spores, stresses the influence of varying environmental conditions, such as humidity, pH, and age, on the thermal death point, and concludes that the latter does not represent a fixed value. However, with such an organism as *E. gingivalis*, it is seen that environmental conditions in the test tube and in the human mouth can, of necessity, vary from each other only slightly. In other words, the thermal death point for a given strain does rather closely approximate a fixed value here, since the amoeba will live only in a relatively small pH range, and since it must be surrounded by a liquid favorable for growth. Also, the culture medium is very similar to the environment in which the amoebae are found in the mouth, with respect to the concentration of salts and of the animal protein present.

We have found, as will be seen later, that the greatest source of variation in reactions to a rise or fall of temperature is in the age of the culture (number of transplants since isolation), and that strains from different sources react similarly.

While it seemed impossible that an amoeba in the vegetative state could resist drying, experiments were carried out to determine whether or not even slight desiccation would kill the organism, and some interesting results were noted. The technique was as follows:

Small coverglasses were cleaned and placed in petri dishes and the whole sterilized in the dry air oven. One drop of a 48-hour culture showing a good growth of amoebae was placed on the coverglass, the lid of the petri dish was lifted so as to allow a slight circulation of air, and the drop allowed to dry. The length of time during which the drop of culture was exposed to the drying process was counted from the moment when no moisture was visible on the coverglass. At the end of this time, the cover with the dried culture was removed from the petri dish with sterile forceps and dropped into a tube of media warmed to 37°. Incubation was at 37° and examinations for growth of amoebae therein were made at the end of twenty-four and forty-eight hours.

TEMPERATURE RELATIONS

We have subjected *Endamoeba gingivalis* *in vitro* to temperatures ranging from 0° to 55° C. The temperature interval was 5° for exposure up to 30 minutes. Below 45°, where the amoebae lived longer at the experimental temperatures, exposure was also made for 1, 2, 3, 4, 5, 10, 18, 24, and 48 hours (table 1). After exposure, the viability of the culture was tested by incubation and subsequent subculturing.

It has already been shown that *E. gingivalis* is easily affected by drugs and dyes (Hlowitt, 1926a; Koch, 1926a) and, if so easily penetrated by chemical substances, it seems reasonable that the delicately protected mass of protoplasm might be easily affected by heat.

The method of inoculating and exposing to varying temperatures tubes containing amoebae has been given above. It was found that lethal temperatures and exposures were as follows:

0°	18 hours	35°	no lethal exposure
5°	24 hours	40°	no lethal exposure
10°	48 hours	45°	20 minutes
15°	no lethal exposure	50°	15 minutes
20°	no lethal exposure	52°	10 minutes
25°	no lethal exposure	55°	2 minutes
30°	no lethal exposure		

A culture was considered viable if only one amoeba was found alive at the given temperature.

These results are given in more detail in table 1. At the higher temperatures, *E. gingivalis* is killed in two minutes at 55°, in ten minutes at 52°, in fifteen minutes at 50°, and in twenty minutes at 45° (fig. 1). Thus it is seen that, as might be expected, *Endamoeba gingivalis* is more sensitive to heat than are the vegetative forms of bacteria found accompanying it in the mouth. Our results are suggestively similar to those of Cleveland (1926) on the lethal effects of heat on the flagellates of the digestive tract of termites.

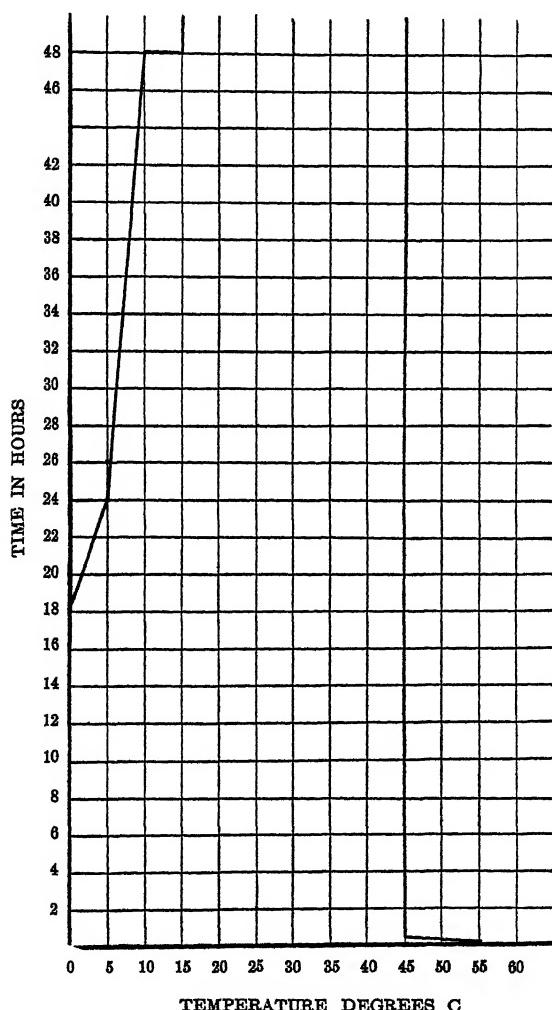


Fig. 2. Graph showing effects of prolonged exposures of *Endamoeba gingivalis* to varying temperatures.

The most striking results, however, were obtained with low temperatures. The amoeba is, apparently, remarkably resistant to temperatures lower than the optimum. It has always been supposed that parasitic Protozoa are very susceptible to temperatures below that of the host, and when such parasites are cultivated, great care is taken to maintain an optimum temperature at all times. There is little doubt that weaker organisms are killed when exposed to sudden changes, such as a reduction in temperature, and that care must be taken to inoculate a new culture into a medium having approximately the warmth of the body, but, on the other hand, the majority of the amoebae withstand these sudden changes, and, if returned to a proper temperature within a reasonable length of time, reproduce normally. Boeck and Drbohlav (1924) note that a stay of more than twenty-four hours in the ice box always resulted in the death of *E. dysenteriae*.

In this connection some interesting facts concerning *E. gingivalis* may be noted. During the course of the present work our strain 38, at that time under cultivation for about sixteen months, was used along with the more recently isolated (two weeks) strain 60, and was found to be more resistant to temperatures below the optimum. At 15° a few amoebae were found to be alive at the end of forty-eight hours. In other words, *E. gingivalis* readily adapts itself to variations in environmental conditions. This is no more than would be expected when one considers the normal habitat of the amoeba and the sudden temperature changes to which at least some of these organisms must be exposed in the mouth. This has been noted by Ilowitt (1925). It is of importance, then, to give the age of the culture when describing experimental work. It is well known that, in the field of bacteriology, many organisms are rapidly altered in their biochemical and biological reactions after undergoing artificial cultivation, and this is also probably true of Protozoa.

In the experiments here cited, for temperatures below the normal, there was no marked inhibition of growth of amoebae above that shown in the control tubes until the lethal temperature was nearly reached. That is, there is apparently no gradual falling off in numbers as the length of time of exposure to a given temperature is increased. This might be taken as an indication that there is only a slight individual variation in resistance among organisms. As an example, the figure for the 5° curve may be cited (table 1). An exposure of twenty-four hours was fatal to all the amoebae at this temperature. Eighteen hours reduced the number markedly, but all shorter exposures had little

effect. If the growth curve for *E. gingivalis* subjected to varying temperatures is plotted, we obtain, not a typical biological curve, but one with a very sudden change between 40° and 45°, and a very gradual change from 20° to 0° (fig. 2). Boeck (1921) noted a similar phenomenon with the cysts of *Iodamoeba bütschlii* where "a great increase in the number of dead cysts occurred at 56° C. over the number of dead at 54° C. Less than one-half as many dead cysts were found at 54° C. as were found at 56° C."

Pantin (1924), in his work on amoeboid movement, has shown that the rate of movement does not increase in a smooth curve, but that there are certain threshold points at which activity is markedly increased or decreased. Inasmuch as movement here seems to depend, according to recent investigators, upon the proportion of the sol phase to the gel phase, and as we find no processes more indicative of vitality than movement and reproduction, it seems only reasonable to suppose that there might be a great similarity between the effects upon the two of certain physical and chemical agents.

MOISTURE RELATIONS

Through experiments upon the effect of drying culture material containing motile *E. gingivalis*, it was found that the organism is unable to withstand a total absence of moisture. While cultures exposed at room temperature *apparently* survived drying, this result was produced because an almost microscopic drop of moisture is able to keep the amoebae alive for a very short interval, and if minute particles of media are present, the organisms within these particles survive three minutes' exposure on an apparently dry coverglass. The amoebae can be recovered by culture methods at the end of this time. Five to ten minutes' exposure yielded no growth. This time was probably sufficient to allow evaporation of all moisture contained in particles of egg in the media. There was no growth in any tube upon repetition of the experiment where special care was taken to remove all particles of coagulated egg visible to the naked eye.

Endamoeba gingivalis is therefore able to withstand, for at least three minutes, drying which is apparently complete when examined with the naked eye, but the slightest bit of moisture is sufficient to enable the amoeba to remain in a living state.

DISCUSSION

The resistance of amoebic cysts to heat has been recorded by a number of workers. Boeck (1921a), testing the viability with eosin, considered the thermal death point to be 68° C. for *E. dysenteriae*. Yorke and Adams (1926), who have succeeded in culturing cysts, and who use this method of determining resistance, obtained no growth of *E. dysenteriae* after exposure of five minutes to 50° C.

It has been found by us that the thermal death point of motile phases of *E. gingivalis* *in vitro* is, for the strains studied, 52° (fig. 1). This temperature holds for strains newly isolated and therefore not modified by prolonged cultivation. Our strain under long cultivation (sixteen months) withstood lower temperatures (15° for forty-eight hours) than did the strain used, which was only about two weeks old. With a thermal death point of 52° it would therefore be safe to say that immersion in water at 60° or above is a rapid and convenient method of sterilizing utensils, instruments, towels, and the like against *E. gingivalis*.

With an organism living under such varied conditions as does this amoeba of the mouth, one would expect to find that its adaptability to its environment would be great. *Endamoeba gingivalis* will remain alive after being exposed to a temperature of 0° for eighteen hours. Boeck and Drbohlav (1924) note that a stay of twenty-four hours in the ice box always resulted in the death of *E. dysenteriae*. The ordinary ice box is kept at about 5° C., and the difference (table 1) between our results and the above may be due to a difference in the age of the cultures. The amoeba will survive temperatures of 20°-40° - that is, from room temperature to a little above body temperature, indefinitely, but is rapidly killed at 55°, and will not withstand temperatures between this and 45° for any great length of time (table 1). It is interesting in this connection to note the experiments of De Rivas (1926) on *E. dysenteriae*. He has recently published some work on the effect of temperature upon various Protozoa, and finds that *E. dysenteriae* is killed by exposure to a temperature of 45° for five to ten minutes. *Endamoeba gingivalis* will survive for twenty minutes at this temperature. Thus, as would be expected, an amoeba adapted to a mode of life which is limited as to temperature variations, as is *E. dysenteriae*, is more sensitive than is *E. gingivalis*. However,

De Rivas' experiments were done with material as obtained from the patient. This was placed in a test tube and kept at the desired temperature. In such experiments, where the amoebae were not given a favorable environment for survival, it would seem that the variation in bacterial flora, acidity, age, and number of amoebae would give inconclusive results, but, on the whole, that the lethal temperature for *E. dysenteriae* might be higher if all other antagonistic factors were eliminated. Boeck (1921) found a marked difference between the length of survival of washed and unwashed cysts. How much greater must be the effect of accumulated metabolic products of surrounding bacteria upon vegetative forms!

Whether those rises in temperature above the normal which cause death are lethal because of coagulation of the protoplasm or because of a change of the rate of sol to gel phase, with consequent disturbance of the equilibrium (Pantin, 1926; Schwitalla, 1925), or whether because of an increase in the metabolic rate, with consequent rapid "wearing out" of the protoplasm before division can take place (Tanner and Wallace, 1925), is not known.

If the most striking of the above facts, that is, great resistance to low temperatures, and great resistance to apparent drying are considered together, it may be concluded that *E. gingivalis* is easily transmitted by "droplet infection," and that any minute amount of saliva carrying the organisms, even though it be microscopic, may be infectious if not completely dry. It is possible then to spread the organisms by the use of the common drinking cup, spoon, and like utensil, as well as by direct contact.

SUMMARY AND CONCLUSIONS

For the strain of *Endamoeba gingivalis* studied,

1. Lethal temperatures and exposures are as follows:

0°	18 hours	30°
5°	24 hours	35°
10°	48 hours	40°
15°	45°	20 minutes
20°	50°	15 minutes
25°	55°	2 minutes

2. The thermal death point is 52° C.

3. Older strains are slightly more resistant than those newly isolated.

4. The amoeba is very sensitive to high temperatures, and the transition from a lethal to a non-lethal temperature is rapid, a stay of twenty minutes killing all amoebae at 45°, while at 40° survival is possible for an indefinite length of time.

5. Immersion in water at 60° C. or above is a rapid and convenient method of sterilizing against *E. gingivalis*.

6. The most minute quantities of liquid or solid material carrying amoebae, if moist, may be infectious.

7. From this we may conclude that *E. gingivalis* may be spread both by direct contact and by an intermediate contaminated article.

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Transmitted June 15, 1927.

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BY

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UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 4, pp. 31-51

Issued October 4, 1927

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

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H. CORWIN HINSHAW

INTRODUCTION

Trichomonas buccalis is a common parasite of the human mouth (Hinshaw, 1926b). It was first successfully grown *in vitro* by Lynch (1915), who used acid bouillon as a culture medium. His results were unsatisfactory, and he was unable to maintain the parasites for an indefinite period of time *in vitro*. Ohira and Noguchi (1917) cultivated this parasite with considerable success, using a mixture of ascitic fluid and Ringer's solution. This medium was not sufficiently selective, for transplants were necessarily made every twenty-four hours when the cultures were maintained at 37° C. We have maintained this parasite for seven weeks at 37° C. without transplanting. Again, in 1922, Lynch reports culturing the *Trichomonas* of the mouth in a variety of protein solutions, including dilutions of blood serum, ascitic fluid, ovarian cyst fluid, and pleural fluid in physiological saline. Hogue (1926) maintained cultures of this parasite on sheep serum saline solution mixed with saliva. When covered with paraffin oil, single cultures were maintained for as long as 30 days without transplanting.

ACKNOWLEDGMENTS

The author is grateful to Professor C. A. Kofoid, under whose direction these studies were made. Dr. G. W. Simonton and Dr. F. V. Simonton gave great aid in the collection of material from cases of pyorrhea. Dr. K. F. Meyer suggested the use of pancreatic digest of casein as a culture medium and gave many other valuable and much appreciated suggestions. A grant from the California Stomatological Research Group helped to make the work possible.

CULTURE MEDIA

1. *Boeck's medium (modified by Howitt, 1925)*

(a) Supernatant fluid.

NaCl	0.9 g.	Locke's solution
CaCl ₂	0.2 g.	
KCl	0.42 g.	
NaHCO ₃	0.2 g.	
Glucose	0.251 g.	
Distilled H ₂ O	1000 g.	

Whites of 1 or 2 eggs
N/20 HCL, 20 cc.

(b) Solidified slants.

8 fresh eggs
Locke's solution (as above), 100 cc.

In the preparation of the supernatant fluid, the Locke's solution was first prepared and sterilized in the autoclave for 20 minutes at 10 pounds' pressure. The egg whites were then removed aseptically from the eggs previously sterilized by 15 minutes' immersion in 70 per cent alcohol. If sterile rubber finger cots are placed on the thumb and forefinger of each hand, it is possible to prepare sterile media without filtration. In this case the whites of two eggs were utilized for each liter of Locke's solution. Otherwise only one egg was used and the fluid filtered through a large Berkfeld candle.

The slants were prepared by coagulating the mixture, placing the tubes in a slanted position in the autoclave and sterilizing for 30 minutes at 15 pounds' pressure with all valves closed.

This medium quickly deteriorates at room temperature, becoming alkaline. The addition of any considerable amount of acid to neutralize destroys the salt balance. If kept in an electric refrigerator at a temperature near 0° C. it will remain fresh for several days.

2. *Locke's, blood serum, egg slant medium--*

(a) Supernatant fluid.

Locke's solution (as in No. 1), 100 cc.
Rabbit blood serum, 10 cc.

(b) Solidified slants. (Same as in No. 1.)

This medium is much more stable than No. 1, but the labor and expense involved in procuring the serum make it less desirable.

3. Locke's, egg albumen, blood serum, egg slant medium—**(a) Supernatant fluid.**

Locke's solution, 1000 cc.

White of one egg

Rabbit blood serum, 1 drop to each 2 cc.

(b) Solidified slants. (Same as in No. 1.)

This medium gives the most profuse growth of any of the mediums that we have used. We have computed as many as 800,000 to each cubic centimeter of medium. The blood serum is added aseptically to each tube just before inoculation. If the serum be introduced at the base of the slant it will remain as a separate, slowly diffusing layer constituting a source of reserve food supply and greatly prolonging the life of the culture. This is an excellent medium for maintaining stock strains. If a layer of heavy paraffin oil be placed on top of the supernatant fluid, the multiplication of aerobic bacteria will be sufficiently inhibited to permit the flagellates to survive for as long as seven weeks.

4. Locke's, egg albumen, blood serum medium—

Locke's solution, 1000 cc.

White of one egg

Rabbit blood serum, 1 drop to 1 cc.

This medium, with no slant of coagulated egg, is capable of maintaining only very meager growth when compared with No. 1, No. 2, or No. 3. Cultures seldom last more than one week and never reach a count higher than 10,000 per cc. It is similar to that utilized by Hogue (1926).

5. Locke's, casein digest, egg slant medium—**(a) Supernatant fluid.**

Locke's solution, 100 cc.

10 per cent casein, tryptic digest, 5 cc.

(b) Solidified slants. (Same as in No. 1.)

The tryptic digest of casein was prepared according to the method given by Kristensen, Lester and Jürgens (1925), except that we prepared smaller quantities and used technical casein instead of commercial casein. The method is as follows: Add 100 g. of technical casein to 1000 cc. of tap water. Stir constantly while bringing to the boiling point. Add 5N NaOH to pH 8.5-9.0 (about 25 cc.). The sodium salt of casein thus formed is soluble, forming a milky fluid. Cool to 37° C. before adding 0.5 g. desiccated trypsin (Grübler). To prevent the growth of microorganisms, 7.5 cc. of chloroform are added.

The flask is now closed with a cork to prevent the evaporation of the chloroform and is placed in an incubator adjusted to 37° C. The flask should be shaken each day for the first three or four days. The milky fluid contained in the flask tends to precipitate after a few hours, leaving a clear yellow supernatant fluid. The precipitate gradually decreases as the digestion continues. When about half of the precipitate is digested the process, which takes from seven to nine days, may be stopped. Then add 20 cc. concentrated hydrochloric acid diluted in 100 cc. of water. Boiling five minutes will leave only a very fine flocculent precipitate. The solution is now filtered through filter paper and the filtrate made up to volume (1000 cc.). This should be distributed in small flasks, each containing enough for one lot of culture medium. This acid stock solution may be kept for several weeks in the ice box, but if it is to be kept longer, 0.5 per cent chloroform should be added. When the medium is desired for use the entire contents of one flask should be boiled 10 minutes to drive off the chloroform and to dissolve the crystals of certain amino acids which have collected. Sodium hydroxide is now added until the reaction is at the neutral point (pH 7.0). For the cultivation of parasitic Protozoa the stock solution is diluted with a balanced saline solution, as stated above.

6. Locke's, casein digest medium—

Locke's solution, 100 cc.

10 per cent casein, tryptic digest, 10 cc.

In this simple medium fair growth of *Trichomonas buccalis* takes place. The maximum number recorded was approximately 30,000 per cc. Better cultures were obtained but were not counted. On some occasions superior results were obtained with a 0.5 per cent solution of digested casein instead of a 1 per cent solution. This is probably largely dependent upon the bacterial flora present.

The principal advantage of the casein medium lies in its stability. It will keep indefinitely in acid stock solution. Furthermore, it can be sterilized in the autoclave in the tubes, insuring a sterile unvarying medium. Media containing fresh protein, such as egg albumen or blood serum, are difficult to procure and to retain readily in a sterile state. Digested casein may be boiled shortly before inoculation to provide a proper fluid for the growth of anaerobic Protozoa. It is seldom that two lots of any other culture medium give the same results in rate of multiplication, etc. The casein digest medium may be made up in large quantities, sufficient for several months, thus

lasting through the entire research problem if desired. Although the medium as now constituted does not yield as prolific a growth as certain other media, the advantages noted make it highly desirable, especially in attempts to rid cultures of contaminating bacteria.

7. *Locke's, protein, living tissue*—

Any of the above media can be greatly improved by the addition of pieces of sterile tissue. Several of the culture media used in bacteriology require sterile tissue. This usually involves the sacrifice of many animals, and even under ideal conditions contamination may result, as animal tissues are not always sterile. We have found that tissue obtained from chick embryos serves our purpose admirably, and, because of its cheapness and the facility with which it is used, we believe that it should have wider application, especially in the cultivation of anaerobic bacteria.

Clean, fertile eggs are incubated for ten days or longer. A pencil mark should indicate the side that is to be kept uppermost in the incubator. The embryo will develop on the upper side and the mark will identify its position should adherence to the shell prevent its free rotation. The following manipulations are best carried on inside a well lighted, clean box with a glass front or top through which operations may be viewed. Protected arm holes are devised to permit free motion of the hands without admitting air currents carrying dust and spores of microorganisms. If necessary a small atomizer may be utilized to spray the air of the interior and lay the dust particles. A good supply of sterile forceps and scissors, each instrument wrapped separately, should be available. The shell of the egg is sterilized by immersion in 70 per cent alcohol for thirty minutes. With a pair of heavy sterile forceps the portion of shell covering the air cell at the large end of the egg is removed. A second pair of forceps can be utilized to pierce the membrane and grasp the embryo by the neck, removing it without touching the shell if possible. If there is any doubt of the sterility of the embryo, it should be rinsed in several changes of sterile Locke's solution or other balanced saline solution. Otherwise it is well not to wash the embryo as the fluid in which the embryo is bathed seems to be a valuable addition to the culture medium. Dissection of the embryo is now carried on with sterile scissors and forceps. Small pieces of tissue may be removed and added aseptically to tubes of culture media. For most purposes the entire embryo may be cut up with the scissors and added to the bottom of culture tubes in 0.5 cc. quantities. For special purposes

different organs serve differently. For instance, in our preliminary experiments liver tissue seemed to contain a growth accelerating agent. It is the only medium in which we have ever succeeded in getting *Trichomonas buccalis* to grow in a hanging drop in air, although dozens of attempts have been made with other media. On the other hand, nerve tissue seems to depress the rate of multiplication and lengthen the life of cultures several weeks. Other experiments in this interesting field are under way.

TECHNIQUE

To express correctly the influence of various factors upon the multiplication and longevity of cultures, it is obviously essential to devise a method for the accurate quantitative enumeration of the number of organisms present in any culture tube at any time. In the scanty literature upon the subject there has appeared, to our knowledge, no accurate quantitative method. Another method of numerically estimating the Protozoa in culture is the one instituted by Howitt (1925), used by Kofoid and Wagener (1925), and Koch (1926). This method consists of removing a portion of the sediment from the tube, placing some of it upon a slide, covering with a cover-glass, and counting the number of organisms observed in a certain number of fields. This method was inadequate for the present work because of the large number of variables involved. The size of the lumen of the pipette tip is an important factor. A tip with an oblique lip which closely clings to the surface of the slant will receive more sediment than one with a square tip. The rapidity with which the material is withdrawn and the exact portion of the slant from which it is obtained are very important factors. Some of these variables are relatively constant with any one experimenter, but the results of one cannot be duplicated by another. We have attempted to use a strictly objective technique which could be duplicated by anyone.

The method which we have found to give excellent results follows. Each culture tube is filled with a measured quantity of the medium, i.e., 10 cc. When multiplication has taken place the contents of the tube are thoroughly agitated. In order that the procedure might be uniform, we filled and discharged the inoculating pipette twenty-five times in quick succession. A small quantity of the fluid is now withdrawn and two large drops are placed in an ordinary haemocytometer

counting chamber. The volume of the chamber and the quantity of fluid in the culture tube being known, the number of organisms in each tube is readily calculated. Successive counts seldom vary more than 10 per cent from the first count if the flagellates are numerous. If less than 10,000 per cc. are found, several mounts must be made.

INFLUENCE OF CERTAIN PHYSICAL AND CHEMICAL FACTORS UPON GROWTH

TEMPERATURE

Trichomonas buccalis is a true parasite adapted to the body temperature of its host. This flagellate can best be cultivated continuously *in vitro* at temperatures close to 37° C. When exposed to room temperatures of 20° C. with an approximate variation of $\pm 3^{\circ}$ C., cultures will survive for three to six days depending upon the number of organisms in the tube at the beginning of the experiment.

TABLE 1

AVERAGE SURVIVAL OF *Trichomonas buccalis* AT ROOM TEMPERATURE

Original number	24 hours	48 hours	72 hours	96 hours	120 hours
69,000	112,000	123,000	58,000	25,000	0

The original number was obtained by the inoculation of 2000 organisms into a tube containing 10 cc. of medium No. 1 (egg slant, Locke's, albumen) followed by incubation for 24 hours at 37° C. The agitation of cultures for the counting procedure was found to have an inhibitive effect, so undisturbed cultures were counted in each case. It will be observed that multiplication is continued for at least 24 hours, resulting in an approximate duplication of the original number. This number survives for 24 hours longer, on the average, and then the number rapidly declines. The control tubes, remaining at 37° C., continued multiplication, showing 980,000 per tube after 120 hours of incubation.

If tubes of fresh media kept at room temperature are inoculated with relatively small numbers of flagellates no multiplication can be observed, and they seldom remain alive for more than 48 hours. This is due, at least in part, to the fact that the culture medium unaltered by bacterial activity is not a favorable medium for the growth of the flagellates.

When incubated cultures are placed in an electric refrigerator with thermostat control adjusted to maintain a temperature of $10^{\circ}\text{ C}.$ with a variation of $\pm 1^{\circ}\text{ C}.$, no multiplication takes place. Cultures so treated give successful transplants for 72 hours, but seldom longer.

If the refrigerator is adjusted to maintain a temperature of $0^{\circ}\text{ C}.$ with a variation of $\pm 1^{\circ}\text{ C}.$, successful subcultures may be secured after 48 hours exposure. If a drop of culture fluid containing a good growth (75,000 per cc.) of *T. buccalis* be placed on a glass slide, covered with a coverglass, and placed at $0^{\circ}\text{ C}.$ for 48 hours, it will be observed that nearly all motility is lost. However, when removed to $37^{\circ}\text{ C}.$ a partial return of motility is observed.

Cultures were placed in an electric water bath with an accurately controllable thermostat with a variation of $\pm 0.1^{\circ}\text{ C}.$. On such occasions two tubes were immersed simultaneously. One of these bore a thermometer and the other was used as a source for transplants to a series of tubes kept at $37^{\circ}\text{ C}.$ When the thermometer indicated that the fluid in the tubes had attained the temperature of the bath we considered that the interval of exposure had started. Only two temperatures have been well studied at the present date. The figures represent averages.

TABLE 2

SURVIVAL OF *Trichomonas buccalis* AT $45^{\circ}\text{ C}.$

Number of minutes exposed	3	4	5	6	7	8	9	10	11	12	13
Growth of subcultures	+	+	+	+	+	+	\pm	\pm	\pm	-	-

TABLE 3

SURVIVAL OF *Trichomonas buccalis* AT $47.5^{\circ}\text{ C}.$

Number of minutes exposed	5	6	7	8	9	10	11	12
Growth of subcultures	+	\pm	=	\pm	-	-	-

These results differ from those of Hogue (1926). However, she found a wide difference in the susceptibility of different strains to high temperature.

EFFECT OF DESICCATION

Small narrow strips were cut from No. 1 coverglasses by means of a diamond glass marking pencil. These were sterilized in Petrie dishes in the hot air sterilizing oven. A single drop of a rich culture of *Trichomonas buccalis* was placed upon each of these pieces of glass and allowed to slowly dry at room temperature. At varying intervals in this process of desiccation these cover-glasses were dropped into

tubes containing culture medium. By this method it was found that complete desiccation invariably resulted in death to all the flagellates. When but little precipitated protein was present death often resulted long before the drop was completely dried. Death was probably due to alterations of osmotic pressure produced by evaporation of the solvents in the culture medium. When slimy precipitated protein material is present the trichomonads tend to bury themselves in the protective medium as described in an earlier paper (Hinshaw, 1926a). This enables them to better withstand desiccation. It is likely that in sputum or in viscous saliva a greater amount of desiccation might be tolerated than under the laboratory conditions described above.

The ability of this parasite to withstand desiccation has important bearing on its mode of transmission from host to host.

SURVIVAL IN WATER

Rich cultures of *Trichomonas buccalis* were suspended in twenty times their volume of distilled water. After varying intervals 1 cc. quantities of this suspension were removed and placed in 10 cc. of culture medium No. 1. The results are given in table 4.

TABLE 4
SURVIVAL OF *Trichomonas buccalis* IN WATER

Hours exposed	1	2	3	4	5	6	7	8	9	10	11	12	13	14	24
Growth in subculture ..	+	+	+	+	+	+	+	+	+	+	±	±	—	—	—

The tolerance of this organism to exposures to low osmotic pressures is surprising. When observed under the microscope the cells suspended in water were seen to be completely spherical and highly distended with fluid. Locomotion was slow but regular. The nucleus and the axostyle, with a portion of the cytoplasm, constituted an irregular mass of protoplasm in the gel state and moved about freely in the fluid which constituted the greater bulk of the cell. With each stroke of the flagella this mass moved laterally. The fluid contents of the body contained refractile granules exhibiting marked Brownian movement. The nuclear membrane was remarkably sharp and clear cut. The pellicle of the body was quite distinct. The pellicle was evidently sticky, for considerable numbers of bacteria adhered to its outer surface. This extreme swelling of the cells was evidently not lethal, for excellent subcultures were obtained.

From these observations it is evident that transmission from host to host can be readily effected through the agency of contaminated drinking cups or other means whereby aqueous solutions of saliva are ingested.

LIGHT

Several experiments were performed with ultraviolet light and our conclusions are still incomplete as to its mode of action.

If a small drop of a rich culture of *Trichomonas buccalis* be placed on a slide, ringed with vaseline, and covered with a quartz cover-glass, a relatively anaerobic chamber is made, although some oxygen undoubtedly dissolves in the medium during transfer. Such preparations were exposed to the radiant energy emitted from a water cooled quartz mercury arc lamp of the Kromayer type operating on 110 volts direct current and consuming approximately 5 amperes. The light was transmitted to the preparation through a quartz rod applicator one-quarter inch in diameter, 6½ inches long, which was placed in contact with the outlet window of the lamp at one end while the rounded end was touching the surface of the quartz coverglass overlying the flagellates. The trichomonads remained partially active for as long as two hours. If a drop from the same culture be suspended from the undersurface of the same coverglass in a hollow ground slide containing air, a slight lessening of motility is observed within two minutes; after five minutes there is considerable lessening of motility, and within seven minutes practically every organism is immobilized. To determine more definitely whether the oxygen was responsible for the differences observed the following apparatus was devised. A high continuous ridge of hard paraffin was constructed around the cavity in a hollow ground slide. Small capillary glass tubes were heated and imbedded in either side of this ridge, so as to form passageways into the interior of the chamber when the latter was covered with a quartz coverglass. A hanging drop of culture fluid containing large numbers of *Trichomonas buccalis* was prepared on the underside of a quartz coverglass and this was placed so as to cover the chamber. Hydrogen gas was now quickly admitted to the chamber for several minutes through one of the capillary tubes. The other tube served as exit for the displaced air. When the air was well displaced with hydrogen the tubes were sealed with melted paraffin and the organisms now under partly anaerobic conditions were subjected to the action of ultraviolet light. Under these circumstances trichomonads

remained active for from forty to fifty minutes. It thus appears that under our conditions of experimentation the lethal effect of the ultra-violet light is dependent upon the presence of free oxygen. It may be that peroxides are formed in the culture medium and that these are lethal to the flagellates. More investigation is required to settle this point.

RADIUM

Through the kindness of Dr. Emil Beck we were enabled to perform a single preliminary experiment on the effect of radium emanations upon *Trichomonas buccalis* in culture. The steel capsules and needles containing the radium were placed in small sterile glass vials floating in the culture medium. The results are shown in table 5.

TABLE 5
THE EFFECT OF RADIUM UPON *Trichomonas buccalis*

Tube No.	Amount of radium contained	Number inoculated	Number in 24 hours	Number in 48 hours	Number in 72 hours
1	50 mg.	81,000	103,000	122,000	191,000
2	37½ mg.	81,000		none	none
3	25 mg.	81,000	few	few	very few
4	10 mg.	81,000	few	none	none
5	Control	81,000		66,600	none
6	Control	81,000		none	none
7	Control	81,000		-	526,000

Since several of the controls were negative we cannot say that the death of the flagellates in tubes No. 2 and No. 4 was due to lethal action of the radium. However, the experiment shows us the one thing that we wanted to know, namely, that this parasite will tolerate doses of radium emanation which in a much shorter time would necrotize human tissue. Therefore, it could not be used in attempts to rid a host of this infection. The flagellates in tube No. 1 actually multiplied in the presence of 50 mg. of radium. The various tubes were placed in a row in the incubator so that each was to a certain extent influenced by the radiations of the others.

OXYGEN

The work of Cleveland (1925) indicated that free oxygen might be very toxic to all forms of parasitic Protozoa. With the possible idea of utilizing oxygen *in vivo* for ridding a host of this parasitic infection we attempted the following experiments *in vitro*. Oxygen

was bubbled through cultures containing *Trichomonas buccalis* for varying periods of time. The oxygen was in some cases run directly from a cylinder of the compressed gas through a rubber tube and sterile glass pipette into the cultures. In a number of other experiments in which we attempted to learn the effect of the rate of flow, we stored and measured the oxygen in a 10-liter graduated bell jar, and removed it through the culture by negative pressure from a suction pump attached to the water faucet. Although several weeks were spent in this phase of the work, very discordant results frequently occurred in spite of well controlled conditions of experiment. Usually the resistant cultures contained fair amounts of débris of precipitated protein in which the flagellates might retreat and be protected from the effects of the gas. If oxygen be bubbled through culture tubes containing 10 cc. of supernatant fluid in which many trichomonads are growing (i.e., 100,000 per cc.) for thirty minutes at a rate of 100 cc. of oxygen per minute, no living flagellates are likely to be observed twenty-four hours later. After forty-eight hours of subsequent incubation a few active trichomonads will likely be seen. If the treatment with oxygen is repeated at this time, all the flagellates will probably perish. It is usually necessary to bubble oxygen through the cultures for 60–80 minutes if all the trichomonads are to be destroyed at one treatment. Cultures of *Trichomonas buccalis* will readily withstand two or three hours of similar treatment if air is substituted for oxygen, and almost unlimited treatment when pure hydrogen is used.

When living in cultures with bacteria, *Trichomonas buccalis* is in a strictly anaerobic environment. This can be demonstrated by the addition of methylene blue which is reduced to the leucobase. Furthermore, this trichomonad is probably an obligate anaerobe. The experiments with oxygen indicate this, but the failure of the organism to survive for any great length of time when placed in a hanging drop is more conclusive. Extensive attempts to cultivate the parasite in hanging drops of culture media have all resulted in failure with the exception noted in an earlier section where mention was made of the fact that excellent cultures were obtained when *Trichomonas buccalis* was placed in a hanging drop containing living chick embryo liver tissue. Extensive attempts to induce growth on the surface of solid media under aerobic and partially anaerobic conditions failed.

HYDROGEN ION CONCENTRATION

The initial hydrogen ion concentration of the culture medium should be slightly alkaline. This seems to offer a more favorable medium for the growth of the bacteria with which *Trichomonas buccalis* is so closely associated. The flagellate will reproduce within a relatively wide range of hydrogen ion concentration. The changes in hydrogen ion concentration taking place in the medium are apparently due almost entirely to the bacteria, and the extent of these changes varies with different strains. Our strain No. 57 was cultivated for several weeks in association with only two morphological types of bacteria. One of these was a very small short chain Gram positive streptococcus and the other was a much larger Gram positive streptococcus growing in very scant numbers. Only the former could be cultivated on plates of blood agar. Before media could be prepared for a biochemical study of the metabolism of these bacteria, the culture of *Trichomonas* became contaminated with several types of cocci and one short Gram negative rod, and the strain has not since been secured free from all of these contaminating organisms. Before this contamination occurred the reaction of the medium (No. 1) would drop to pH 5.6 within the first twenty-four hours of cultivation regardless of the initial pH. The reaction remained near this point throughout the life of the culture. These cultures seldom lasted longer than seven days and growth was never abundant. If only a fraction of a drop was inoculated the trichomonads very frequently would fail to grow, although bacterial growth would take place. In these tubes the alteration in pH would almost exactly parallel that of the tubes containing trichomonad growth, showing that the observed change in reaction was largely due to activities of the bacteria.

After the strain became contaminated the same early shift to the acid reaction was observed, followed by a shift in the reverse direction during the second twenty-four hours of growth. The medium would become alkaline, reaching pH 7.6-7.8. This shift in the alkaline direction was possibly due to the activities of a small Gram negative bacillus which had contaminated the cultures. Cultures made on blood plates during the shift in the acid direction showed relatively few colonies of this organism. When the reaction was becoming alkaline, greatly increased numbers of the colonies of rods appeared on the plates.

As is mentioned in a later paragraph, the omission of carbohydrates from the culture medium resulted in an immediate shift in the alkaline direction without the preliminary development of acid.

The acids involved in the increase of hydrogen ions were not determined beyond the recognition of the characteristic odor of butyric acid which is always present in our cultures.

PROTEINS

As shown in the section on media, a number of proteins or their split products may serve for the nutrition of *Trichomonas buccalis*. Proteins from hen's egg were used most of the time because of the ease with which they could be obtained. The albumen of the hen's egg alone is insufficient to support this organism. Several experiments, each utilizing a wide range of concentration of this protein, gave entirely negative results. The addition of raw yolk to the albumen solution did not permit as luxuriant a growth as when the yolk is coagulated. Egg yolk alone does not support the growth of this organism. Proteins derived from the living tissues of chick embryos serve very well for the food of *Trichomonas buccalis*. Lynch (1915) was able to cultivate these organisms for a short time upon acidified bouillon. Ohira and Noguchi (1917) preferred the use of ascitic fluid diluted with equal parts of Ringer's solution. It is probable that none of these proteins are utilized in their native state, but that the products of bacterial decomposition must constitute the main item of diet of *Trichomonas buccalis*. At least the presence of bacteria is required, and haemocytometer counts demonstrate that no multiplication of trichomonads takes place during the first seven to nine hours after transplantation, during which time the bacteria are multiplying.

The simplest medium in which this organism grew well was medium No. 6, composed of Locke's balanced saline solution and pancreatic digest of sodium caseinate. The protein was largely split to amino acids in this instance and in consequence growth was at a maximum within twenty-four hours and declined after two or three days.

CARBOHYDRATES

Several sugars have been incorporated in various media with satisfactory results. In the presence of several types of bacteria these were probably promptly split. For that reason we were unable to draw any important conclusions from these experiments.

Trichomonas buccalis can multiply in protein media containing little or no carbohydrate. If the glucose be omitted from the formula for Locke's solution in medium No. 1 or in medium No. 6, the resulting medium will support growth of this parasite. The most striking thing about such an experiment is the fact that the medium quickly becomes alkaline. This "protein sparing action of carbohydrates" is a phenomenon widespread in nature. When carbohydrate is present the bacteria and Protozoa will utilize this substance largely for their source of energy. When the supply is exhausted proteins are attacked. The deamination of the amino acids during the process of protein metabolism results in the production of basic substances which reduce the concentration of hydrogen ions.

INFLUENCE OF CERTAIN BIOLOGICAL FACTORS UPON *TRICHOMONAS BUCCALIS*

BACTERIA

All our evidence indicates that there is a close relationship between *Trichomonas buccalis* and the bacteria with which it is associated in culture. Furthermore, there are certain types of bacteria which are accelerative and many types which are distinctly depressive in their mode of action upon the multiplication of this flagellate. It was mentioned in an earlier section that the strain of *Trichomonas buccalis* which we maintained for a time in association with relatively few types of bacteria was not particularly vigorous. Furthermore, the number of bacteria present was relatively small. After four or five days of growth the supernatant fluid of the culture tube was only very slightly turbid with bacterial growth. Under these circumstances cultures of the trichomonad usually died out within ten days or less. The maximum number of flagellates observed was less than 75,000 per cc. and was often less than half this amount. Accidental

contamination of the media in which this strain was cultured took place and in the following transplant 669,000 trichomonads were computed to be present in each cc. of the culture fluid. We could trace this remarkable acceleration of growth to no other factor except the presence of these additional species of bacteria. The second transplant yielded 800,000 flagellates to each cc. of culture media. This number gradually diminished in successive transplants returning after several weeks to an average level of about 100,000 protozoans per cc.

Extensive efforts, consuming several months, were made to rid these cultures of Protozoa of all bacteria. Every attempt has failed and we doubt if they are capable of living in any of our present media without the presence of bacteria.

Our attempts at mechanical segregation of Protozoa and bacteria followed two lines. The first of these and the most promising is their individual isolation by means of an original micropipette apparatus to be described in another paper. We have isolated scores of trichomonads, washed them entirely free of bacteria, and have planted them in several types of media, but with no results. Not only have we made use of various fresh media, but we have passed rapidly multiplying cultures through a Berkfeld filter, and planted individual specimens of *Trichomonas buccalis* in these partially exhausted media with no positive results.

Following the suggestion of Andrews (1926) we tried the use of Norton alundum filters. The RA84 grade of alundum filter has pores sufficiently small to hold back many trichomonads, but sufficiently large to allow the passage of many bacteria. It was found, however, that a sufficient number of bacteria were always retained to give a luxuriant growth when subcultures were made.

Ultraviolet light was found to kill the flagellates before the bacteria were harmed.

Cultures containing mainly coccus forms of bacteria were inoculated with cultures of *Bacillus pyocyanus*, which has well known powers of inhibiting the growth of cocci. The cocci were inhibited to a certain extent, but the flagellates were equally inhibited. Neither were exterminated by the treatment. The filtrate from cultures of *B. pyocyanus* had a similar effect.

It was mentioned above that certain bacteria have an accelerative effect upon the growth of *Trichomonas buccalis* in culture. Many other bacteria are decidedly antagonistic to the growth of this proto-

zoan in culture. The intensely proteolytic and putrefactive bacteria are especially potent in this regard. Some of these have the power of breaking up the coagulated egg slant, others cause a darkening of the surface of the slant. These are probably anaerobic organisms.

TISSUE CULTURES

Attempts were made to grow *Trichomonas buccalis* in symbiosis with tissue culture cells instead of in symbiosis with bacteria. The tissue cultures were made according to the well-known technique of Lewis and Lewis (1924). The explants were of 10-day chick embryo tissues, preferably of liver tissue. After incubation for twenty-four hours in an inverted position, in order to secure a fair outgrowth of tissue cells on the coverglass, a single specimen of *T. buccalis*, washed free of bacteria by means of our micropipette apparatus, was planted in the tissue culture. The trichomonad can survive and retain fair activity among the tissue cells and in the absence of bacteria for one or two hours. The flagellate finally tends to become abnormal, frequently casts off half or more of its cytoplasm by the process of autotomy described in an earlier paper (Hinshaw, 1926a), and eventually dies without reproducing itself. If bacteria together with trichomonads are planted in a culture of chick embryo liver cells, a flourishing but short-lived growth of the Protozoa will often take place.

The addition of pieces of sterile, living, chick embryo cells to cultures of *Trichomonas buccalis* is often beneficial, as described in the section on technique.

SUMMARY

1. Several culture media have been devised which give excellent growth of *Trichomonas buccalis* *in vitro*.
2. *T. buccalis* will survive a temperature of 0° C. for 48 hours; 10° C. for 72 hours; room temperature for 3-6 days; 45° C. for 9-11 minutes; and 47°.5 C. for 7-9 minutes.
3. *T. buccalis* will not survive complete desiccation in dilute aqueous solutions.
4. This organism will withstand suspension in distilled water for 10-12 hours.

5. In the presence of air *T. buccalis* is killed in less than 7 minutes upon exposure to a Kromayer water-cooled quartz mercury arc lamp. In the absence of free oxygen this treatment may be continued for 1-2 hours.

6. *Trichomonas buccalis* may multiply for 72 hours in contact with a capsule containing 50 mg. of radium.

7. Oxygen is toxic for *Trichomonas buccalis* *in vitro*. All parasites directly exposed to the action of the gas are usually killed by 30 minutes of slow bubbling of this gas through the cultures. Forms hidden in débris may escape this lethal action of the gas. This parasite is probably an obligate anaerobe.

8. The *Trichomonas* of the mouth multiplies in a relatively wide range of hydrogen ion concentrations. The activities of the bacteria in the cultures so mask the activities of the protozoan as to make biochemical studies of the latter impossible.

9. The split products of a number of proteins may be utilized by *T. buccalis*. The simplest medium yielding growth was pancreatic digest of sodium caseinate highly diluted with Locke's solution, forming a 0.5-1 per cent solution.

10. Carbohydrates may be omitted from the formulas for culture media without destroying their power to support the growth of this parasite. In the absence of this carbohydrate the reaction of the medium becomes alkaline very quickly.

11. We have not succeeded in cultivating *T. buccalis* free from bacteria although the isolation of this protozoan is not impossible.

12. Contamination of cultures by certain bacteria has resulted in a great acceleration of growth. Many other bacteria are decidedly antagonistic to this flagellate *in vitro*.

13. *Trichomonas buccalis* can be grown in hanging drop tissue cultures of chick embryo liver with bacteria. In the absence of bacteria, radical autotomy and death ensue within two hours.

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INFLUENCE OF THE HYPOPHYSIS
UPON THE THYROID GLAND
IN AMPHIBIAN LARVAE

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UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 4, pp. 53-78, plates 3, 4

Issued October 4, 1927

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

INFLUENCE OF THE HYPOPHYSIS UPON THE THYROID GLAND IN AMPHIBIAN LARVAE

BY

BENNET M. ALLEN

It has long been known that the interrelations of the endocrine glands are complicated and far-reaching, yet in the nature of things the problem is a difficult one. The first to recognize the influence of the hypophysis upon the thyroid gland in amphibians was Adler (1914), who removed the hypophysis of tadpoles, 20 mm. in total length, by cauterization through the roof of the mouth. He showed that in these hypophysectomized tadpoles the thyroid gland remains very small and contains colloid secretion meager in amount and of thin consistency.

A method for removal of the hypophysis (exclusive of the posterior lobe) at its very inception was worked out independently by Allen (1916) and by P. E. Smith (1916), each making observations upon the effect produced upon metamorphosis and upon the correlated functioning of the thyroid gland. These writers showed, as did Adler before them, that the removal of the hypophysis (exclusive of the posterior lobe) prevents the tadpoles from undergoing metamorphosis. Allen and E. R. Hoskins working independently, each without knowledge of the other's work, demonstrated at the same time (1916) that removal of the thyroid gland anlage in *Rana*, resulting in the complete absence of this gland, likewise prevents metamorphosis. These and other experiments make it increasingly clear that one of the physiological units (anterior lobe) of the hypophysis cooperates with the thyroid gland in producing the colloid secretion stored up in the thyroid gland. Preparations of the thyroid gland had been shown by Gudernatsch (1912, 1914) greatly to accelerate metamorphosis while, as we show below, feeding of the hypophysis does not produce this result. Smith (1916) found the thyroid gland of a normal

NOTE.—The work reported upon in this paper was supported in part by a research grant from the University of Kansas; in major part, however, by research grants from the University of California.

control tadpole, 38 mm. total length, to be three times the size of that found in a tadpole, 37 mm. total length, from which the hypophysis had been removed. The latter had fewer vesicles than the control tadpole and there was little colloid secretion in them.

Allen (1917a) found that there was very little difference in the widths, of sections of the thyroid glands of hypophysectomized tadpoles of *Rana pipiens* as compared with those of the normal controls during early stages, 16.5 and 21.5 total length, but those of the tadpoles operated on were only about one-half the thickness of those of the normal controls and contained only scant colloid. Differences in size became greater in later stages.

Smith (1920) in his able monograph upon hypophysectomized tadpoles gave the weights of wax models of operated and control tadpoles. In hypophysectomized tadpoles of 36 mm. or more total length, the thyroid glands were found to have one-sixth the weight of the glands of the normal controls, a result due to retardation in development and colloid secretion.

Allen (1920) reported the results of separate implantation of the anterior, intermediate, and posterior lobes of the adult hypophysis into normal, thyroidless, and hypophysectomized tadpoles. In this article it was stated "The anterior lobe produces a marked acceleration in the development of the hind legs. This happens to be most conspicuous in the hypophysectomized specimens probably because they were the first operated upon, but at the date of writing, June 14, the same appears to be true of the normal and the thyroidless specimens into which this lobe has been transplanted." In a later paper, however (Allen, 1921), it was shown that this developmental acceleration of thyroidless tadpoles was not the result of implantation of the anterior lobe of the hypophysis alone. Those specimens in which metamorphosis had been thus accelerated were cases in which the operation for removal of the thyroid gland had not been successful. This point was easily checked when the tadpoles were killed in November. The specimens in which the thyroid gland had been successfully removed showed no tendency to metamorphose even though the implanted anterior lobe of the hypophysis was fully maintained for several months previous to the time of killing. It was shown that metamorphosis is due to the combined action of both the anterior lobe of the hypophysis and the thyroid gland.

At this same time, Swingle (1921) made intraperitoneal implants of the anterior lobe of the hypophysis of *Rana catesbeiana*, *R. pipiens*, and *R. clamitans* into immature normal *R. catesbeiana* tadpoles. This

caused acceleration of limb development. Swingle did not, however, make implants into thyroidless and hypophysectomized tadpoles. He also produced metamorphosis by implanting the thyroid gland, in line with the well-known fact that feeding thyroid gland material hastens metamorphosis.

Hoskins and Hoskins (1920) claimed that feeding of the anterior lobe of the hypophysis produces metamorphosis in tadpoles. The writer had produced the same result in 1918 by feeding commercial preparations of anterior hypophysis to tadpoles of *Rana pipiens* and in the following year repeated the experiment by feeding anterior lobe material of cattle, prepared in our laboratory, to *Bufo americanus*. In the latter experiments growth acceleration was brought about but there was not the slightest evidence that any tendency toward metamorphosis was produced. The above personal observations were never published, the writer being convinced that feeding pure anterior hypophysis had no effect upon metamorphosis.

Smith and Cheney (1921) most conclusively showed that the results attained by the Hoskins were due to the fact that the commercial anterior hypophysis material is not pure, having an iodine content 120 times as great as that of pure anterior hypophysis material. With the use of fresh and dried material which they themselves prepared, they did not find any acceleration of metamorphosis.

Smith and Smith (1923) published a most illuminating paper upon "The Function of the Lobes of the Hypophysis as Indicated by Replacement Therapy with Different Portions of the Ox Gland." Their method was to feed the material of the different portions of the gland as they had been cut apart. In addition to this method, experiments were performed in which the fresh glands were ground in sand, washed in saline solution, centrifuged, and injected intra-peritoneally. The significant results are expressed in their own words regarding the influence of the anterior lobe of the hypophysis upon the metamorphosis of hypophysectomized tadpoles. "The feeding of the anterior lobe does not elicit any response from the atypical glands of the albino, actual injection of the substance being necessary in order to effect their repair. These effects on the thyroid from the injections of bovine anterior hypophyseal substance are in accord with transplantation experiments in amphibia (Allen, 1920, Swingle, 1921)."

Allen (1920) had previously shown that implantation of the anterior lobe of the hypophysis into hypophysectomized tadpoles causes a resumption of metamorphosis that would otherwise have practically ceased, and further, showed that this is accompanied by the

enlargement of the thyroid gland together with a rich accumulation of colloid substance in the follicles. It was thus made clear that the production of colloid by the thyroid gland is made possible by the functional activity of the anterior lobe of the hypophysis. Smith's injection experiments mentioned above probably provided a sufficient amount of the essential secretions of the anterior lobe of the hypophysis to produce the same result. It was shown (Allen, 1917b, 1925) that removal either of the thyroid gland or of the hypophysis causes the larva to cease metamorphosis at precisely the same stage. This was the conclusion drawn from measurements of the hind-limb anlagen of a large number of tadpoles collected through a series of years.

As stated above, the thyroid gland remains very poorly developed in tadpoles from which the hypophysis, exclusive of the posterior lobe, has been removed. We have, in the implantation of different parts of the hypophysis, a method for determining just which part is concerned in this cooperation with the thyroid gland. This line of work is most tedious and we have spent several years in accumulating the data presented in this paper. The work was begun at the University of Kansas, but by far the greater part of the data were gathered during the last four years in Los Angeles. The forms studied were *Rana pipiens* and *Bufo americanus* in Kansas, and *R. aurora draytoni* (the California red-leg frog) and *B. boreas halophilus* in California. Both of these forms breed here in January, so it is quite possible to make an early start in the year's work.

TECHNIQUE

Implantations were made into both normal tadpoles and those from which the hypophysis had been removed from six weeks to two and a half months previously. The implantations were made under the integument, always at one point—just over the right eye. By observing this uniformity, it was possible to dissect out the implant to see whether it had been maintained or had been resorbed. If a second implant was made it was placed over the left eye.

It might be well to go a little farther into the method of implantation. The tadpoles, at a length of about 20 to 25 mm., are anaesthetized by placing them in water in which a few drops of ether has been thoroughly mixed. The glandular substance to be implanted is obtained from adult frogs that are quickly killed by decapitation. The roof of the skull is removed, the brain lifted up, and the hypophysis removed and placed in boiled water. The instruments used are

sterilized by immersion in alcohol. It must be admitted, however, that these precautions are rather superfluous and of value chiefly because of their subjective influence upon the conscience of the experimenter. Complete asepsis is quite out of the question because the moist, delicate skin of the tadpole cannot be sterilized and the wound does not heal until an hour or so after the tadpole has been put back into its aquarium. It is idle to think of keeping the water in which it lives sterile. Faeces are constantly being passed by the tadpoles, especially after operations upon them. That this technique is successful is shown by the very striking and specific effects produced by the implantations and by the fact that the implanted glands dissected out months after the implantation not only have escaped resorption but also are shown by microscopic study of sections to have undergone little change. We hope eventually to give an account of the histological character of these implanted glands. Further check was made upon this point in some cases by killing the glandular material before implantation through immersion in boiling water or a week formaldehyde solution. In these cases, the implanted glands were completely resorbed within two weeks and had no appreciable effect upon the tadpole.

In all of the hypophysectomized tadpoles, the brain was dissected out and a careful search made to determine whether the hypophysis had been completely removed. The cases in which removal had not been complete were classed as "unsuccessful attempts" and used as one type of control.

In all tadpoles, measurements of trunk length, i.e., from tip of snout to cloaca, were compared with total length. In each case special attention was given to limb length which serves as a good basis for estimating the degree of metamorphosis. The age of the tadpoles was estimated from February 15, which is an average date for the operations for removal of hypophysis or thyroid gland. Slight deviations from this counted so little in the total duration of the experiment that they were not taken into account.

Measurements of the thyroid glands were made by dissecting out in each case the hyoid cartilage and completely exposing the thyroid glands. This could readily be done by using a binocular microscope. Outline drawings were made with camera lucida and the areas computed by means of a planimeter. Three kinds of determinations were made: (1) the flat ventral surface; (2) the end view as seen from the caudal end; (3) the diameter of the follicles. The end view was not so accurate as the ventral view for several reasons. In the first place

the hyoid cartilage with the thyroid glands was stood on end and held in position under water in a Syracuse dish between two small pieces of glass. In some cases the piece was no doubt slightly inclined, thus giving a distorted view. Then there was the difficulty of clearly distinguishing the contour of the end view when seen by reflected light and drawn with the use of a camera lucida. Another cause for error was found in the fact that the right and left glands do not lie quite parallel but are inclined toward one another. As a result, the camera lucida projection had the character of a diagonal optical section. Allowance for this was made by pointing off with dividers upon the drawing of the end view, the actual maximum width as seen in the ventral view. The end view was then re-drawn free-hand in its correct width. This method impartially applied gave fairly reliable results. Extreme accuracy is made unnecessary, however, because of the decisive character of the results.

Necessarily very crude determinations were made of the size of the follicles. They are small, dimly seen in the thicker thyroid glands, and the method involved the arbitrary choice of two or three of the largest follicles of each gland. They are given for what they are worth. Fortunately the results are so decisive that they have value only as accessory findings. The illustrations of thoroughly representative glands give the most conclusive interpretation of these values of follicle size.

While it might have been desirable to estimate the volumes of these irregular bodies, in order to give the results mathematical finish, such a procedure would give a false appearance of accuracy; but above all it would be superfluous. It is felt that the points that we are investigating are sufficiently demonstrated by the agreement of the three lines of evidence.

Camera lucida drawings of typical thyroid glands as seen in ventral view and of the same glands as seen in transverse section serve further to support and illustrate the differences in the thyroid glands of the various types of extirpation and implantation combinations.

SUMMARY OF AVERAGES

TABLE 1

Duration of implantation	Age in days	Total length mm	Body length mm	Leg length mm	Flat surface mm	Thyroid Gland		Area mm	End mm	Follicle mm	
						Area	Right				
<i>Rana aurora draytoni</i>											
83	37 1	15 3	1 24	114	034	005	119	034	006		
137	58 2	18 6	16 6	287	134	012	263	117	013		
202	50 5	19 1	14 8	267	095	012	284	113	016		
		Control		First Series (13)							
		Control		Second Series (11)							
		Control		Third Series (6)							
		—Hypophysis Attempt (unsuccessful).		First Series (3)							
		83 40 1		19 1 2 0		041	008	105	044	007	
		—Hypophysis Attempt (unsuccessful)		Second Series (14)							
		171 28 5		22 7 24 6		399	186	017	413	018	
		—Hypophysis Attempt (unsuccessful)		+Intermediate Hypophysis (4)							
89	121	59 1	20 8	7 8	200	100	010	229	099	011	
		Control		+Anterior Hypophysis (3)							
31	132	34 0	18 7	14 0	273	121	041	246	116	011	
		Control		+Intermediate Hypophysis (7)							
31	142	41 2	17 8	4 3	168	046	010	159	040	011	
		Control		+Posterior Hypophysis (5)							
39	139	43 8	17 9	7 9	161	054	006	189	069	007	
		—Hypophysis.		First Series (7)							
		83 39 1		18 2 0 6		080	017	002	085	018	002
		—Hypophysis		Second Series (8)							
		147 48 7		16 6 65		070	019	002	078	018	002
		—Hypophysis		Third Series (8)							
		195 45 0		20 0 1 1		138	026	003	141	026	003
		—Hypophysis		Fourth Series (15)							
		393 58 1		24 2 1 7		210	053	004	227	054	004
		—Hypophysis		+Anterior (dead). (4)							
		149 41 9		15 7 0 9		103	036	004	094	035	003
		—Hypophysis		+Anterior. First Series (5)							
38	124	53 8	18 9	14 9	214	094	014	200	064	010	
		—Hypophysis		+Anterior Second Series (13)							
79	125	57 5	18 9	17 9	270	111	016	270	116	017	
		—Hypophysis		+Intermediate (8)							
76	142	49 7	17 2	0 7	083	023	003	093	024	003	
		—Hypophysis		+Posterior (4)							
73	122	42 0	13 0	0 4	051	016	001	060	011	002	
		—Hypophysis.		+Posterior +Anterior (4)							
29	128	60 7	19 9	8 1	281	099	013	287	117	016	
		—Thyroid. (10)									
		126 54 5		0 9							
		—Thyroid		+Anterior Hypophysis. (7)							
58	118	57 7			1 1						

TABLE 1—(Continued)

continued) Thyroid Gland
Area: Right

Area: Left

Duration of implantation	Age in days	Total length mm.	Body length mm.	Leg length mm.	Flat surface mm.	End mm.	Follicle mm.	Flat surface mm.	End mm.	Follicle mm.
<i>Rana pipiens</i>										
—Thyroid (unsuccessful). +Anterior Hypophysis. (4)										
116	212	32.1	18.8	18.1						
					—Thyroid. +Anterior Hypophysis. (6)					
153	239	47.0	19.2	0.8						
<i>Bufo boreas halophilus</i>										
—Hypophysis. (13)										
	242	42.8	16.8	2.43	116	.053	.004	.112	.047	.003
					—Hypophysis. +Anterior. (7)					
	331	43.7	17.0	7.7	.274	.115	.008	.277	.118	.009
					—Hypophysis. +Intermediate. (2)					
	248	37.2	15.3	2.1	.085	.032	.011	.113	.036	.006
					—Hypophysis. +Total Hypophysis. (5)					
	318	38.6	15.7	8.5	.208	.137	.011	.334	.151	.010
					—Hypophysis. +Hypophysis (boiled). (3)					
	326	39.8	18.0	2.8	.133	.051	.003	.128	.046	.005
					—Thyroid. (8)					
	291	49.0	19.4	2.2						
					—Thyroid. +Total Hypophysis. (6)					
42	301	50.4	19.4	2.7						
					—Thyroid. +Anterior. (14)					
58	268	44.9	17.9	2.4						

TABLE 2

Rana aurora

Area: Right Thyroid gland

oid gland Ventr. Left

Dur. of imp.	Date killed	Total length mm.	Trunk length mm.	Leg length mm.	Flat surface mm.	End mm.	Follicle mm.	Flat surface mm.	End mm.
First Series of Controls. (13)									
5/9	27 1	10 5	0 33	.050	.010	.0031	.010	.018	.002
5/9	31.3	12 6	0 6	.086	.018	.002	.073	.017	.004
5/9	33.8	13.5	0 9	.069	.027	.007	.060	.023	.006
5/9	36 0	14 3	1 2	.093	.036	.000	.097	.034	.006
5/9	35 4	14.5	1 0	.097	.034	.005	.079	.030	.003
5/9	33 6	14 5	0 8	.053	.020	.003	.065	.023	.005
5/9	39.5	15 0	1 0	.147	.037	.007	.100	.028	.007
5/9	36 9	15 7	0 0	.072	.014	.003	.083	.034	.004
5/9	39 1	16 3	1 0	.097	.018	.005	.103	.025	.006
5/9	42 3	17 3	1.5	.143	.064	.007	.157	.038	.011
5/9	39 6	17.6	1.8	.105	.060	.007	.073	.063	.007
5/9	44.8	18.9	1.8	.135	.063	.005	.165	.067	.008
5/9	43.8	18 2113	.048	.010	.140	.051	.018
Av. 83 days ...		37.1	15.3	1.24	.114	.0341	.005	.119	.0343
	± .7385	± .4284	± .0982	± .0064	± .0083		± .0071	± .0084	
σ.....	3.948	2.282	.489	.0340	.0187		.0884	.0343	
	± .5222	± .3018	± .067	± .0045	± .0022		± .0050	± .0208	
C. V.	10.64	14.91	39.43	30.35	49.06		32.26	46.00	
	± 1.407	± 1.972	± 5.428	± 4.014	± 6.489		± 4.267	± 6.084	

Rana aurora—(Continued)

Second Series of Controls. (11)

Dur. of imp.	Date killed	Total length mm.	Trunk length mm.	Leg length mm.	Thyroid gland			Area: Left		
					Area: Right			Flat surface mm.	End mm.	Follicle mm.
6/20	51.2	19.0	23.1	.353	.200	.018	.317	.140	.019	
6/20	58.0	19.0	25.1	.356	.189	.013	.363	.187	.016	
6/20	56.5	18.0	20.4	.424	.207	.011	.283	.167	.017	
6/6	52.3	17.4	7.7	.250	.052	.013	.200	.053	.009	
6/20	61.0	19.0	18.0	.313014	.330016	
6/6	58.3	16.6	7.4	.230	.093	.007	.170	.057	.009	
6/20	59.0	18.4	15.4	.217	.081	.013	.207	.075	.009	
6/20	56.5	18.9	18.5	.240	.107	.012	.277	.088	.013	
6/20	63.0	19.1	16.4	.263	.125	.010	.233	.147	.007	
6/20	60.7	18.5	12.5	.233	.138	.011	.217	.103	.013	
6/20	64.3	21.0	18.0	.273	.153	.009	.297	.157	.010	
Av. 137.45 days	58.2	18.6	16.6	.287	.134	.012	.263	.117	.013	
	± 7.86	± .3375	± 1.092	± .0128	± 0.106		± .0119	± .0110		
σ	3.869	1.288	5.365	.0631	.0501		.0587	.0517		
	± 5.63	± .1852	± .7715	± .0080	± .0075		± .0084	± .0077		
C. V.	6.647	8.908	31.93	21.98	37.38		22.319	44.17		
	± .9558	± 1.281	± 4.591	± 3.160	± 5.637		± 3.208	± 6.681		

Third Series of Controls. (8)

8/21	51.1	18.2	17.3	.367	.093	.014	.334	.101	.020
8/21	55.6	18.7	12.2	.257	.105	.014	.317	.147	.014
8/21	50.9	18.9	15.8	.240	.120	.012	.271	.101	.014
8/21	53.4	19.0	18.9	.308	.074	.015	.343	.130	.022
8/21	44.3	10.6	14.0	.173	.077	.007	.200	.103	.010
8/21	47.5	20.0	10.4	.257	.097	.011	.241	.099	.013
Av. 202 days..	50.5	19.1	14.8	.287	.095	.012	.284	.113	.016
—Hypophysis attempt (unsuccessful). First Series. (3)									
5/9	37.2	18.5	1.9	.135	.050	.010	.110	.043	.009
5/9	88.1	18.3	1.4	.157	.030	.006	.113	.033	.008
5/9	45.0	20.6	2.7	.142	.035	.008	.093	.055	.005
Av. 83 days....	40.1	19.13	2.0	.145	.041	.008	.105	.044	.007

—Hypophysis attempt (unsuccessful). Second Series. (14)

8/27	22.2	20.7	24.6	.400	.241	.017	.441	.193	.025
8/27	29.3	21.2	27.3	.320	.147	.013	.310	.127	.014
8/28	21.7	21.7	29.0	.433	.207	.025	.476	.200	.027
8/1	25.8	21.8	27.5	.300	.117	.013	.458	.169	.016
7/20	26.7	22.0	22.5	.383	.138	.013	.427	.187	.017
8/3	29.1	22.4	25.7	.377	.203	.019	.370	.153	.015
8/28	39.6	22.6	29.5	.402	.173	.030	.387	.203	.025
8/21	28.3	22.8	29.5	.383	.201	.017	.391	.253	.019
8/1	24.7	22.7	22.0	.507	.168	.013	.430	.180	.011
8/1	33.0	23.0	30.0	.447	.150412	.253	.011
7/20	29.3	23.6	29.3	.443	.200	.017	.353	.170	.016
8/1	25.0	23.6	31.2	.327	.147	.012	.417	.118	.015
7/8	27.7	24.4	27.4	.353	.236	.021	.457	.270	.017
7/12	36.1	24.5	30.6	.513	.382	.012	.453	.217	.023
Av. 171.28 days	28.5	22.7	27.6	.399	.186	.017	.413	.192	.018

Rana aurora—(Continued)

—Hypophysis attempt (unsuccessful). +Intermediate Hypophysis. (4)

Dur. of imp.	Date killed	Total length mm.	Trunk length mm.	Leg length mm.	Thyroid gland			Area: Left		
					Area: Right			Area: Left		
96	6/24	62.5	20.0	6.48	.220	.133	.008	.243	.143	.009
98	6/26	57.0	19.5	12.20	.213016	.240012
77	6/5	57.5	20.8	6.08	.213	.080	.009	.280	.080	.013
84	6/10	59.3	23.0	6.50	.155	.087	.007	.155	.067	.011
89		59.1	20.8	7.81	.200	.100	.010	.229	.090	.011

Av. 121.25 days from Pit. operation.

Control. +Anterior Hypophysis. (3)

33	6/25	41.6	16.3	13.7	.343	.120	.100	.225	.083	.007
51	6/22	23.7	19.6	23.1	.307	.187	.022	.303	.220	.020
10	5/21	36.8	19.8	5.2	.168	.055	.007	.120	.047	.005
31		34.1	18.6	14.0	.273	.121	.041	.246	.116	.011

Av. 132.3 days.

Control. +Intermediate Hypophysis. (7)

33	6/25	42.4	16.4	1.6	.140	.023	.007	.127	.041	.006
30	6/22	47.8	16.5	2.9	.138	.024	.008	.173	.027	.009
33	6/25	32.3	16.7	2.4	.150	.050	.009	.120	.030	.010
30	6/22	43.9	17.0	3.6	.200	.053	.007	.130	.040	.008
30	6/22	43.0	18.1	2.6	.143	.042	.005	.160	.043	.012
30	6/22	44.0	18.4	3.0	.167	.051	.013	.180	.030	.012
30	6/22	48.9	21.8	13.1	.235	.079	.017	.212	.070	.018
31		43.2	17.8	4.3	.168	.046	.010	.159	.040	.011

Av. 142.85 days.

Control. +Posterior Hypophysis. (5)

42	6/22	40.0	15.9	1.7	.007	.027	.003	.067	.027	.002
33	6/25	40.5	17.4	14.1	.231	.080	.006	.220	.107	.007
36	6/7	41.0	17.6	4.5	.113	.037	.008	.263	.065	.008
42	6/22	47.0	18.7	4.2	.173	.043	.007	.165	.036	.006
42	6/22	50.5	19.7	15.2	.220	.083	.008	.233	.110	.008
39		43.8	17.0	7.9	.101	.054	.006	.189	.069	.007

Av. 139.6 days.

—Hypophysis. First Series. (7)

5/9	37.4	17.2	0.6	.074	.010	.001	.100	.020	.001
5/9	37.7	17.5	0.4	.100	.017	.002	.083	.015	.002
5/9	37.1	17.8	0.5	.060	.015063	.017
5/9	38.7	17.8	0.6	.053	.007	.001	.067	.009	.003
5/9	40.4	18.4	0.8	.073	.022	.001	.073	.018	.002
5/9	43.1	19.0	0.7	.105	.027	.002	.114	.021	.003
5/9	39.3	19.7	1.0	.097	.023	.003	.093	.028	.004
Av. 83 days	39.1	18.2	0.6	.080	.017	.002	.085	.018	.002

Rana aurora—(Continued)

—Hypophysis. Second Series. (8)

Dur. of imp.	Date killed	Total length mm.	Trunk length mm.	Leg length mm.	Area: Right			Thyroid gland			Area: Left		
					Flat surface mm.	End mm.	Follicle mm.	Flat surface mm.	End mm.	Follicle mm.	Flat surface mm.	End mm.	Follicle mm.
	7/14	44.0	15.5	.48	.071	.018	.002	.088	.022	.002			
	8/6	49.9	16.3	.80	.100	.020	.003	.110	.024	.004			
	6/20	45.556	.059	.013	.001	.060	.010	.001			
	6/20	45.2	15.9	.72	.073	.020	.001	.047	.013	.002			
	7/1	48.2	16.1	.64	.080	.022	.001	.064	.017	.001			
	7/1	57.6	17.4	.88	.044	.016	.001	.073	.022	.001			
	8/6	48.0	17.5	.48	.053	.020	.003	.090	.016	.003			
	8/1	51.2	17.3	.64	.103	.027	.003	.093	.023	.001			
Av. 147.75 days		48.7	16.6	.65	.070	.019	.002	.078	.018	.002			

First and Second Series. —Hypophysis were united in computation.

Av. day 115.38

Av.	44.2	17.4	.623	.075	.0184		.081	.0183					
	± 1.008	± .2038	± .0301	± .0035	± .00095		± .0031	± .00088					
σ.....	5.79	1.131	.1733	.0201	.0055		.018	.0051					
	± .713	± .1441	± .0213	± .0024	± .00067		± .0022	± .00062					

C. V.....

C. V.....	13.09	6.507	27.81	26.06	29.89		22.45	27.81					
	± 1.6110	± .829	± 3.424	± 3.275	± 3.681		± 2.758	± 3.423					

—Hypophysis. Third Series. (8)

8/21	38.0	17.2	0.6	.183	.021	.004	.083	.017	.002				
8/21	36.2	18.5	0.8	.087	.017	.002	.100	.013	.002				
8/21	41.0	18.9	0.8	.087	.021	.004	.119	.023	.003				
8/21	43.9	19.8	1.0	.168	.023	.005	.147	.019	.004				
8/21	45.4	20.3	0.9	.117	.033	.004	.177	.037	.003				
8/21	43.5	20.4	0.9	.120	.022	.002	.132	.019	.002				
8/21	53.1	20.9	2.0	.150	.038	.004	.157	.053	.002				
10/24	58.8	24.2	1.5	.267	.035	.002	.217	.026	.002				
Av. 195 days	45.0	20.0	1.1	.138	.026	.003	.141	.026	.003				

—Hypophysis. Fourth Series.

Dur. of imp.	Date killed	Total length mm.	Trunk length mm.	Leg length mm.	Area: Right			Thyroid gland			Area: Left		
					Flat surface mm.	End mm.	Follicle mm.	Flat surface mm.	End mm.	Follicle mm.	Flat surface mm.	End mm.	Follicle mm.
	3/24	46.7	18.4	1.5	.111	.034	.006	.120	.032	.006			
	3/24	53.9	20.9	0.9	.117	.015	.002	.213	.023	.003			
	3/24	46.4	21.0	1.0	.133	.023	.005	.145	.040	.006			
	1/3	50.2	22.7	1.5	.313	.051	.011	.233	.043	.005			
	3/24	57.0	23.1	1.8	.157	.039	.003	.195	.053	.002			
	3/24	56.1	23.4	1.6	.160	.047	.003	.171	.039	.004			
	3/27	55.5	23.4218	.051	.004	.148	.071	.004			
	3/24	52.7	23.9	.7	.136	.045	.003	.170	.039	.003			
	3/24	62.4	24.9	1.7	.193	.040	.002	.273	.043	.002			
	1/26	63.3	25.1	2.1	.165	.059	.003	.250	.063	.005			
	3/24	56.9	25.3	1.6	.216	.065	.005	.209	.070	.004			
	3/24	66.3	25.8	1.9	.269	.057	.004	.322	.061	.004			
	3/24	66.8	27.9	1.9	.300	.053	.004	.323	.052	.006			
	3/24	67.3	28.1	3.5	.290	.090	.008	.310	.092	.005			
	3/24	68.6	29.4	2.4	.370	.128	.008	.320	.098	.008			
Av. 393 days		58.1	24.22	1.7	.210	.053	.004	.227	.054	.004			
		± 1.246	± .5602	± .0018	± .01004	± .0045		± .0116	± .0037				
σ.....		7.158	3.217	.0879	.0577	.026		.067	.021				
		± .8815	± .3861	± .0083	± .0081	± .0032		± .0082	± .0025				
C. V.....		12.32	14.17	3.994	27.14	49.62		29.51	38.88				
		± 1.517	± 1.744	± .491	± 3.33	± 6.107		± 3.62	± 4.790				

Rana aurora—(Continued)

Dur. of impl.	Date killed	Total length mm.	Trunk length mm.	Leg length mm.	Flat surface mm.	End mm.	Follicle mm.	Thyroid gland		
								Area: Right	Area: Left	—
—Hypophysis. +Intermediate. (8)										
28	7/30	49.5	17.7	0.8	.097	.019	.003	.067	.017	.001
84	7/10	45.4	..	0.5	.073	.022	.003	.080	.015	.004
105	7/2	59.5	19.0	0.0	.085	.028	.002	.070	.030	.001
62	7/3	47.9	15.5	0.8	.092	.020	.003	.073	.021	.002
76	7/10	48.2	17.6	0.8	.117	.023	.001	.125	.035	.002
126	7/23	57.4	..	0.6	.073	.014	.003	.103	.016	.003
70	8/6	48.0	18.4	0.5	.113	.030	.005	.161	.033	.006
55	5/12	41.7	14.9	0.7	.090	.017	—	.063	.017	—
Av.										
76	142 days	49.7	17.2	0.7	.093	.023	.003	.093	.024	.003
—Hypophysis. +Posterior. (4)										
98	6/24	50.9	18.0	0.7	.073	.019	.001	.110	.013	.003
77	6/3	44.9	12.5	0.5	.046	.017	.002	.050	.011	.001
91	6/19	36.0	11.6	0.2	.047	.011	..	.018	.007	..
26	6/23	38.4	12.0	0.3	.036	.017	.002	.063	.014	.001
Av.										
73	122 days	42.0	13.0	0.4	.051	.016	.001	.060	.011	.002
—Hypophysis. +Anterior (dead). (4)										
7/15	40.6	13.0	0.5	.070	.027	.003	.068	.016	.003	..
6/24	39.6	15.5	0.6	.067	.016	.004	.083	.017	.003	..
7/20	48.5	18.0	1.1	.143	.053	.006	.133	.061	.005	..
7/18	39.2	16.3	1.4	.131	.048	.003	.090	.047	.002	..
140	41.9	15.7	0.9	.103	.036	.004	.094	.035	.003	..
days										
—Hypophysis. +Anterior. First Series. (Four to six weeks implantation). (5)										
32	8/1	64.9	22.4	15.5	.242019	.267	..	.016
33	6/30	56.5	18.6	15.5	.267	.122	.015	.350	.105	.009
41	6/24	50.8	18.7	11.4	.200	.078	.013	.180	.040	.012
40	6/10	47.8	..	22.7	.214	.093	.009	.137	.069	.005
43	5/1	49.3	18.0	9.5	.147	.083	.013	.067	.041	..
Av.										
38	124 days	53.8	18.9	14.9	.214	.094	.014	.200	.004	.010
—Hypophysis. +Anterior. Second Series. Over six weeks implant. (13)										
55	7/8	60.0	22.0	13.5	.345	.065	.012	.340
55	6/25	57.0	18.4	20.0	.280	.077	.015	.231	.103	.014
55	6/25	61.0	18.0	17.0	.246	.101	.011	.267	.117	.014
59	6/18	50.0	19.0	20.5	.305	.283305	.187	.012
63	6/8	45.3	16.9	16.5	.267	.073	.011	.267	.076	.012
78	6/15	54.2	18.9	22.0	.250	.071	.017	.233	.133	.017
85	6/22	58.3	19.0	18.8	.277	.103	.010	.237	.087	.017
85	6/23	55.3	18.6	22.0	.233	.146	.013	.200	.140	.013
98	6/24	56.9	19.5	18.6	.253	.087	.010	.306	.083	.007
98	6/25	59.3	19.5	13.9	.307	.130	.011	.280	.163	.014
98	6/25	68.0	19.0	14.0	.267	.096	.028	.304	.100	.030
98	6/25	66.0	21.7	19.5	.309	.131	.030	.346	.127	.021
98	6/26	46.9	15.5	16.8	.203	.084	.012	.200	.083	.021
Class Av.										
79	125 days	57.5	18.9	17.9	.270	.111	.016	.270	.116	.017
"Total"										
A.V.	126 days	56.8	19.0	16.6	.250	.105	.015	.246	.109	.015
		± .883	± .3112	± .527	± .0064	± .0104		± .0088	± .0065	
σ.....		4.725	1.864	2.731	.0346	.0556		.0454	.0334	
		± .6249	± .220	± .381	± .0046	± .0074		± .006	± .0046	
C. V.....		7.34	8.804	15.25	12.81	50.09		16.81	28.79	
		± .9708	± 1.157	± 2.017	± 1.67	± 6.625		± 2.22	± 3.906	

Dur. of imp.	Date killed	Rana aurora—(Continued)						Thyroid gland		Area: Left	
		Total length mm.	Trunk length mm.	Leg length mm.	Flat surface mm.	End mm.	Follicle mm.	Flat surface mm.	End mm.	Follicle mm.	
—Hypophysis. +Posterior. +Anterior Hypophysis. (4)											
20	5/23	57.7	19.2	2.1	.240	.079262	.092	...	
11	7/23	64.8	21.0	7.2	.220	.077	.007	.250	.103	.009	
32	6/24	59.5	20.3	6.8	.263	.117	.017	.316	.140	.027	
55	6/25	61.1	19.0	16.1	.323	.120	.015	.240	.133	.013	
Av.											
29	128 days	60.7	19.9	8.1	.261	.099	.013	.267	.117	.016	
Duration of implantation		Age days	Total length mm.		Leg length mm.		Condition of thyroid		Condition of implantation		
			—Thyroid. (10)								
		130	60.2		1.36		Absent				
		116	56.9		1.00		Absent				
		116	61.7		1.00		Absent				
		116	50.5		1.00		Absent				
		116	58.8		.50		Absent				
		116	56.4		.87		Absent				
		131	47.3		.56		Absent				
		131	50.8		.80		Absent				
		145	53.4		.80		Absent				
		138	49.5		.80		Absent				
Av.		126	54.5		.87						
		—Thyroid. +Anterior Hypophysis. (7)									
56		108	56.0		1.04		Absent		O.K.		
57		109	55.0		.66		Absent		O.K.		
77		129	62.5		.91		Absent		O.K.		
63		115	60.0		1.16		Absent		O.K.		
77		129	60.6		1.00		Absent		O.K.		
56		108	57.4		1.17		Absent		O.K.		
19		127	53.0		1.58		Absent			
Av.	58	118	57.7		1.07						

TABLE 3

Rana pipiens

Duration of implantation	Age days	Total length mm.	Trunk length mm.	Leg length mm.	Condition of thyroid	Condition of implantation
—Thyroid. +Anterior Hypophysis. Unsuccessful in removal of Thyroid as shown by dissection. (4)						
84	181	16.2	16.2	Met.	Single	O.K.
75	172	18.3	17.1	Nearly Met.	Single	Not found
163	249	43.2	19.8	18.4	Single	O.K.
153	249	50.5	22.1	17.9	Single	O.K.
Av.	116.25	32.05	18.8	18.15(2)		
—Thyroid. +Anterior Hypophysis. (6)						
150	249	63.8	26.3	1.35	Absent	Fair
150	50.6	20.7	.79	Absent	O.K.
154	287	41.2	17.0	.43	Absent	O.K.
154	237	44.0	17.9	.46	Absent	O.K.
154	237	44.2	18.0	1.42	O.K.
154	237	38.1	15.3	.66	O.K.
Av.	152.66	239.4	48.98	19.2	.85	
—Thyroid 1½ years old. +Anterior Hypophysis. (2)						
41	271	84.1	33.8	2.94		
41	271	79.0	30.8	2.57		
Av.	41	271	81.65	32.2	2.76	

TABLE 4

Bufo boreas halophilus

- Hypophysis (13)

Dur of imp	Date killed	Total length mm	Trunk length mm	Leg length mm	Lat surface mm	Arc 1		Right		Arc 2		Left	
						Ind mm	Ovalick mm	Lat surface mm	Ind mm	Ovalick mm	Lat surface mm	Ind mm	Ovalick mm
10/29	40 5	14 0	2 8	137		002		167		002		004	
9/6	40 3	14 8	2 7	076		002		097		037		003	
9/6	40 3	14 8	2 7	107		060		009	033	062		003	
11/11	37 5	15 3	1 8	125		037		003	089	035		002	
11/8	40 1	16 2	2 2	083		017		003	083	035		002	
11/8	44 8	16 9	2 2	080		070		004	140	031		004	
11/8	40 2	16 9	2 0	085		067		002	088	060		002	
11/8	44 6	17 0	2 0	112				003	113			002	
9/6	38 6	17 8	2 2	103				001	085			001	
11/8	47 6	17 9	2 0	093		030		008	147	025		005	
11/11	43 0	18 0	2 7	087		022		004	077	023		004	
9/12	50 0	18 4	3 6	090		067		004	140	077		001	
9/13	50 0	21 0	2 7	210		133		013	147	074		003	
Av 242 days	42 8	16 8	2 4	116		053		004		112		047	
Average of right and left				110		050		005					
	± 7546	± 337	± 088	± 0063		± 0059			± 0050	± 0037			
σ	4 034	1 805	073	034		032			03	02			
	± 5335	± 2387	± 0625	± 0044		± 0048			± 0031	± 0030			
C V	9 425	10 74	19 46	31 77	60 37				26 71	42 55			
	± 1 247	± 1 420	± 2 573	± 4 20	± 7 98				± 3 54	± 5 63			
						-Hypophysis	+Anterior	(7)					
1/28	39 0	14 5	5 9	316		106		003	330	111		007	
1/1	39 0	15 5	14 1	273		140		006	320	140		011	
1/27	44 8	16 0	8 5	307		108		015	325	133		021	
1/27	47 0	16 8	4 7	262				004	205			002	
11/3	43 0	17 1	9 3	280		170		009	262	166		006	
1/28	46 0	19 0	7 5	263		100		003	285	100		001	
1/28	47 2	20 0	4 1	220		067		010	211	061		004	
Av 331 days	43 7	17 0	7 7	274		115		008	277	118		001	
Average of right and left				275		117		003					
						-Hypophysis	+Intermediate	(2)					
10/8	32 0	13 1	1 4	103		031		016	083	030		007	
10/24	42 4	17 5	2 8	087		033		006	143	041		006	
Av 248 days	37 2	15 3	2 1	085		032		011	113	036		006	
Average of right and left				099		034		008					
12/21	28 5	13 4	10 7	270		196		013	333	166		011	
12/21	44 0	16 3	6 3	237		150		004	246	153		003	
12/22	32 7	13 9	6 7	259		050		022	360	093		017	
1/28	40 4	17 5	8 3	367		150		010	457	200		011	
12/28	47 5	17 5	10 5	359				005	275			007	
Av 318 days	38 6	15 7	8 5	298		137		011	334	151		010	
Average of right and left				316		144		010					
						-Hypophysis	+½ Hypophysis (bulkd)	(3)					
1/27	36 4	16 5	2 5	147		030		007	069	038		006	
12/22	36 9	17 5	2 7	079		050		001	094	033		002	
1/4	46 0	20 1	3 8	174		072		002	221	067		007	
Av 326 days	39 8	18 0	2 8	133		051		003	128	046		005	
Average of right and left				105		038		005					

Bufo boreas halophilus—(Continued)

—Thyroid (8)

Duration of implantation	Age days	Total length mm	Body length mm	Leg length mm	Condition of thyroid	Condition of implantation
	289	53 5	22 5	2 3	Absent	
	289	55 7	22 9	2 1	Absent	
	263	45 5	17 5	1 4	Absent	
	263	48 6	18 0	2 0	Absent	
	263	42 3	17 5	2 3	Absent	
	263	46 2	18 5	2 2	Absent	
	348	48 2	18 6	2 6	Absent	
	348	52 1	19 8	2 4	Absent	
Av		291	49 0	19 4	2 2	

—Thyroid + Total Hypophysis (8)

37	310	53 0	19 5	2 9	Absent	O K
37	310	50 0	19 0	2 6	Absent	O K
65	257	52 5	18 0	2 9	Absent	O K
37	310	49 7	18 8	2 6	Absent	O K
37	310	45 5	17 0	2 5	Absent	O K
37	309	52 0	24 0	2 8	Absent	O K
Av	42	301	50 45	19 4	2 7	

—Thyroid + Anterior (14)

9	255	47 6	18 0	2 3	Absent	O K
78	270	47 8	19 5	2 4	Absent	O K
79	300	46 7	18 0	2 5	Absent	O K
79	270	46 2	19 0	2 6	Absent	O K
73	276	41 5	16 0	2 0	Absent	O K
42	270	40 5	15 1	2 0	Absent	Small
28	225	44 5	19 5	2 8	Absent	O K
36	294	40 0	17 0	2 0	Absent	O K
23	290	38 5	17 3	2 5	Absent	O K
73	310	45 5	17 0	2 5	Absent	O K
79	270	51 3	19 5	2 6	Absent	O K
79	270	54 0	18 5	2 8	Absent	O K
125	225	45 0	18 7	2 0	Absent	O K
11	233	40 1	18 0	2 6	Absent	O K
Av	58 1	268	44 9	17 9	2 4	

DISCUSSION OF OBSERVATIONS AND RESULTS

Tables 1 to 4 serve to give the numerical data. Table 1 is a summary. Tables 2-4 give the detailed figures.

It is clear that removal of the hypophysis brings about a serious impairment of the growth and functioning of the thyroid glands. So far as our gross measurements and observations go, this retardation in growth of the gland is largely due to the small quantity of stored thyroid secretion—colloid. In these observations the controls show a decidedly greater area of the flat surface of the gland and over double the area of the end as compared with the hypophysectomized tadpoles, this in spite of the fact that hypophysectomized specimens are on the average over 100 days older and distinctly larger, especially as to trunk length. It is of interest to note the very small size of the follicles of the thyroid glands of the hypophysectomized tadpoles as shown in the measurements and in the figures showing surface views and transverse sections of typical cases. In both the control and the hypophysectomized specimens killed on May 9, there were very meager evidences of colloid, although the follicles were well distended with uncoagulable fluid.

The specimens in which there had been an unsuccessful attempt at removal of the hypophysis were on the average 19 days older than the controls. This could hardly account for the fact that they were so much farther advanced in development, as shown by leg length and by shortening of the tail, and it is possible that the unsuccessful attempt at removal of the gland may have stimulated it to greater growth and functional activity. The data are too few to make a decision possible and are of little value for our main problem. But they are valuable for comparison as accessory controls because they serve to show that the poor development of the thyroid glands in the hypophysectomized tadpoles is certainly not due in any sense to mechanical injury but results solely from the complete removal of the hypophysis.

In comparing the older hypophysectomized specimens (killed in July) with the earlier ones, we find that they have undergone further slight development of the thyroid gland and a corresponding increase in the length of the hind legs. This is still more obvious in the group of tadpoles killed over one year after hypophysectomy. In the latter there are numerous small but compact masses of colloid in the thyroid

gland and the hind limbs have attained an average length of 2.14 mm. This development is, however, extremely slow as compared with that of the controls, which completed their metamorphosis six months before under the influence of their own thyroid glands. Even under-fed control tadpoles, in September and October had hind legs of an average length 7 times those of the hypophysectomized tadpoles and thyroid glands of about three times the surface area with follicles of about five times the diameter of those in the oldest hypophysectomized specimens.

In all these experiments the growth of the hind legs can be taken as a clear indication of a tendency toward metamorphosis, but there is a large probable error involved because the growth is so rapid. In a group of tadpoles from the same bunch of eggs and under identical conditions of food, water, and temperature, there is a large amount of individual variation. Limb development may be slow in starting but it often proceeds very rapidly when it does begin. The thyroid glands are rather capricious in their growth and are hard to evaluate correctly. The implanted glands show a great variation in persistence and function. All of these factors, combined with the difficulties of experimentation, dissection of specimens, and observation, make it impossible to conform to the best biometrical standards. The results are, however, so clear-cut that we trust there will be no doubt in the mind of the reader regarding their significance. We give the mean, the standard deviation, and the coefficient of variation in all series where the number of cases in a given class warrants it; but the numbers are nowhere large. Fortunately, there is practically no overlapping of data in specimens of contrasted groups and we do not need to depend upon averages to establish principles which are obvious.

The group of hypophysectomized tadpoles into which the intermediate lobe of the adult hypophysis had been implanted shows characteristics of growth and development altogether comparable with the usual conditions shown in the hypophysectomized tadpoles. As a group they are 100 days younger than the latter and 2.8 mm. shorter in trunk measurement. To this is due the fact that the leg length and dimensions of thyroid glands and follicles all fall distinctly short of those in the control hypophysectomized tadpoles. In all these specimens the implanted intermediate lobe of the hypophysis was in full functional activity as shown by the fact that the tadpoles not only returned to the normal color as a result of the implantation, but became very much darker. The duration of the experiment was such as to test out these points most fully. Compare the leg length and thyroid dimensions of

this group with the corresponding determinations for the controls which were quite comparable as to age and size. It is clear that the intermediate lobe has no influence upon the thyroid gland.

Like conclusions may be made in the case of hypophysectomized tadpoles into which the posterior lobe of the adult hypophysis had been implanted. This has wholly failed, as in the case of intermediate lobe implantation, to restore the thyroid gland to normal size or in any way to induce normal growth.

It may be well to point out here that there were far more operations of these types of intermediate lobe and posterior lobe implants than the above figures represent. A few were discarded because of resorption of the implant or death or injury to the tadpole, and many were continued for weeks without causing any tendency to metamorphosis. Finally implants of the anterior lobe of the hypophysis were made and these in all cases induced further development toward metamorphosis. Unfortunately no accurate measurements of hind leg length were taken at the time of these anterior lobe implants because of the technical difficulties of making reliable and safe measurements of the hind legs. Naturally no measurements of the thyroid glands could be made in the living tadpoles. These experiments were carried out in connection with studies upon the influence of the different lobes of the hypophysis upon size growth to be published after the completion of certain additional experiments this season.

As to the importance of the posterior lobe, this lobe is in all cases present in the hypophysectomized tadpoles although Smith (1920) claims that it does not attain full development in these cases. The implantation serves only to add a greater quantity to that already present.

In marked contrast to these cases of hypophysectomized tadpoles with intermediate or posterior lobe implantation, are those in which the anterior lobe is implanted. The average age of these is far below that of the controls and about one-half that of the hypophysectomized tadpoles without gland implantation. In spite of these facts, the leg length is much greater, 16.4 mm., compared with 9.8 mm. in the normal controls, and 1.3 mm. in the hypophysectomized tadpoles. It will thus be seen that the replacement of the anterior lobe of the hypophysis restores all the features of development that had been inhibited by removal of the anlage that normally produces the anterior, intermediate, and pars tuberalis elements of that gland complex. The greater volume of the implanted gland causes these tadpoles to exceed

normal development. That they do not show as great development as do the tadpoles in which there had been an unsuccessful attempt to remove the hypophysis may be explained by the fact that the average age of the latter exceeds that of the anterior lobe implants by 30.6 days. It is just conceivable that the unsuccessful attempt at removal of the hypophysis had stimulated that gland to greater growth, as mentioned above; but the assumption is not a necessary one.

The most significant feature of this work is the influence exerted by the implanted anterior lobe of the hypophysis in causing an enlargement of the thyroid gland which in these tadpoles is distinctly larger than in the controls. The renewed activity of the thyroid gland may be induced after a practically dormant period of several months. This is shown by the implantations into *Bufo boreas halophilus*, where the control hypophysectomized tadpoles were not so old as the implanted ones but the limb growth of the latter (in the 43 days, average duration of implantation) was so great as to leave no doubt about the influence of the anterior hypophysis implantation. In these cases the thyroid glands of the implants were more than twice as large as those of the controls, as shown in the flat area, end area, and follicle size. These figures apply to the cases where one-half of the anterior hypophysis was implanted. In those in which an entire anterior lobe was implanted, the development of the hind limbs was even further promoted and the cause for this was seen in the still greater size of the thyroid gland.

Colloid masses are found in the thyroid glands in cases where the buccal anlage of the hypophysis had been wholly removed. This can be seen in section (figs. 14, 15, 16). It appears to have a normal consistency especially in the older specimens but is very meager in amount. It is quite clear that amount and quality are not such as to produce metamorphosis, although the hind limb buds do appear only to be arrested in their development. No microchemical study of these glands has as yet been made but it should prove a very interesting and fruitful study. Whether this inability to metamorphose is due to the quantity or quality of the colloid secretion, we cannot say.

A study of the thyroidless tadpoles is interesting. It had long ago been shown that feeding the thyroid gland hastens metamorphosis, as indicated above, and that removal of the thyroid gland inhibits it. Allen (1925) had shown that the degree of hind-limb bud growth is essentially the same in thyroidless, in hypophysectomized tadpoles, and in those from which both of these glands have been removed. This

would appear to indicate that the meager colloid in the thyroid glands of hypophysectomized tadpoles has no influence upon metamorphosis.

The question naturally arose whether metamorphosis could be produced in a thyroidless tadpole by implanting into it the anterior lobe of the hypophysis taken from an adult frog. If the anterior lobe of the hypophysis is so decidedly concerned in metamorphosis as the foregoing facts indicate, could its presence alone produce this process in the absence of the thyroid gland, provided it were of sufficient size? The first tentative observations along this line (Allen, 1920) apparently indicated that it could; but a critical examination of the material showed that in all apparent cases of this sort there had been complete or partial failure to remove the thyroid gland (Allen, 1921). In all cases in which the thyroid gland had been successfully removed, the implanted anterior lobe of the hypophysis utterly failed to induce metamorphosis, or any definite tendency in that direction.

In the tables we give additional data upon this question. As will be seen, this work was carried out upon *Rana pipiens*, *R. aurora draytoni*, and *Bufo boreas halophilus*. In all of these it is equally demonstrated that the anterior lobe of the hypophysis alone without the presence of the thyroid gland will not induce metamorphosis, no matter how great an amount of the anterior lobe of the hypophysis may be implanted.

The growth impulse did not come from secretions stored in the hypophysis or from the additional nourishment resulting from partial absorption of its tissues. This was made clear by implantation of the anterior lobe of the hypophysis after killing it by boiling or by immersion in weak formaldehyde. In each of these lines of experiment, subsequent dissection showed that the entire implant had been resorbed in about ten days without leaving any trace and without exerting any influence toward metamorphosis. In all cases in which implantation of the anterior lobe of the hypophysis resulted successfully, the implant was later found to have persisted intact and to have established a rich blood supply.

CONCLUSIONS

We have here one more line of evidence to show that the process of metamorphosis in anuran larvae is produced by the cooperation of the anterior lobe of the hypophysis working through the thyroid gland. Implants of the intermediate and posterior lobes of the hypophysis do not produce this effect. No experiments were made in the implantation of the pars tuberalis because of difficulties in technique. Whether this would have such an effect we do not know but it is perfectly certain that implantation of the anterior lobe causes marked increase in the deposition of colloid in the thyroid gland accompanied with increase in its size and this results in a tendency toward metamorphosis that is most clearly expressed in the growth of the hind legs.

It is just as clear that the anterior lobe of the hypophysis alone is not capable of inducing metamorphosis, as shown by successful implantations of very large amounts of anterior lobe material from adult frogs into thyroidectomized tadpoles. The thyroid gland is fully as important in metamorphosis as is the anterior lobe of the hypophysis but no more so, as shown in the observations of Allen (1925). Though a slight amount of colloid is stored in the thyroid glands of hypophysectomized tadpoles, this is no more effective in producing metamorphosis than is the hypertrophied anterior lobe of the hypophysis in thyroidectomized tadpoles. Both the thyroid gland and the anterior lobe of the hypophysis are alike essential to any appreciable progress toward metamorphosis.

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EXPLANATION OF PLATES

PLATE 3

Fig. 1. Thyroid glands of *Rana aurora draytoni*, control (ventral view). Killed May 9. Total length 45.8 mm., trunk length 20.0 mm., hind-leg length 2.8 mm. $\times 44$.

Fig. 2. Thyroid glands of *Rana aurora draytoni* control (ventral view). Killed August 21. Total length 53.4 mm., trunk length 19.0 mm., hind-leg length 18.9 mm. $\times 44$.

Fig. 3. Thyroid glands of *Rana aurora draytoni* minus hypophysis plus anterior lobe of hypophysis (ventral view). Implant April 6. Killed June 22. Total length 55.3 mm., trunk length 18.6 mm., hind-leg length 22.0 mm. $\times 44$.

Fig. 4. Thyroid glands of *Rana aurora draytoni* minus hypophysis (ventral view). Killed May 9. Total length 43.1 mm., trunk length 19.2 mm., hind-leg length 0.24 mm. $\times 44$.

Fig. 5. Thyroid glands of *Rana aurora draytoni* minus hypophysis (ventral view). Died October 29. Total length 57.3 mm., trunk length 24.9 mm., hind-leg length 1.1 mm. $\times 44$.

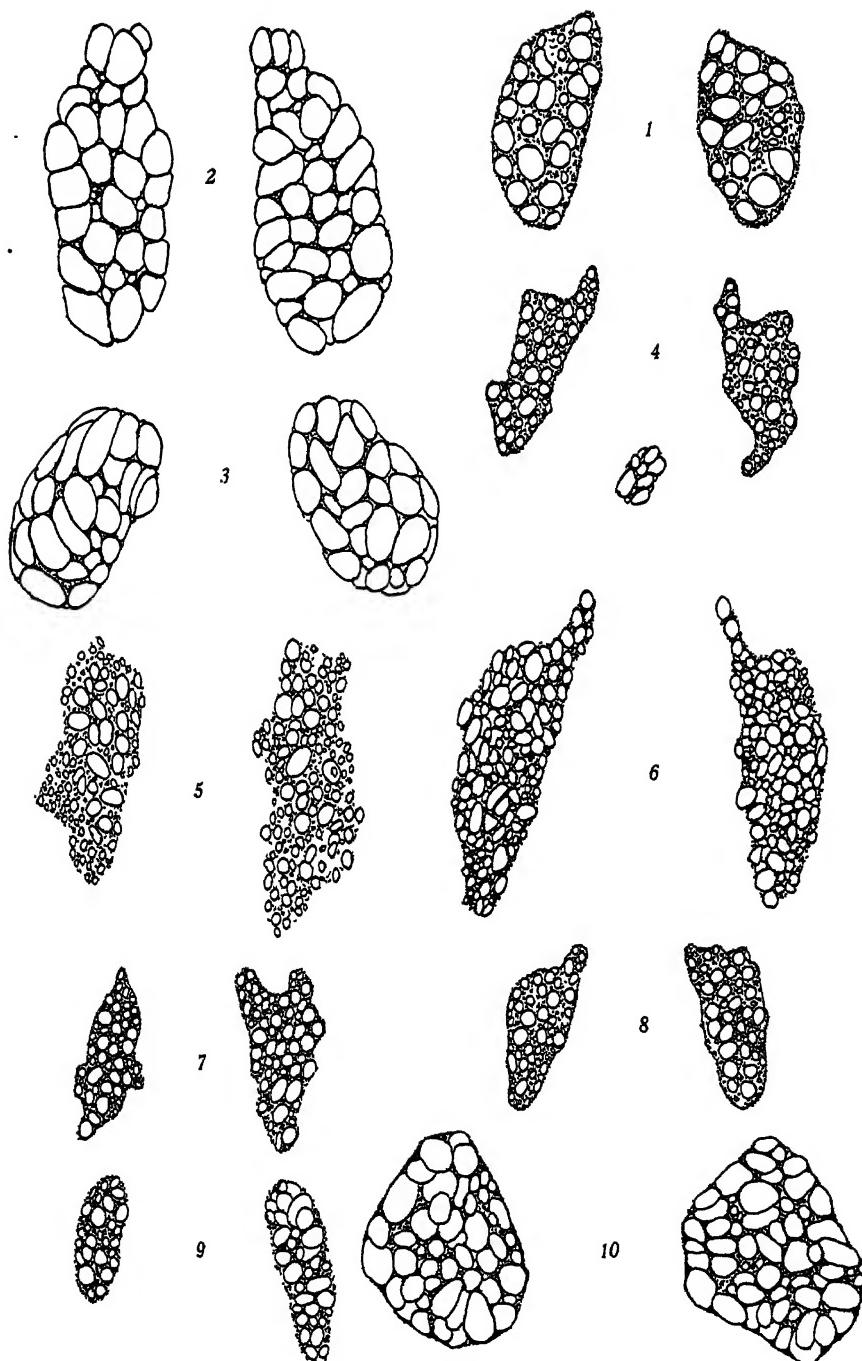
Fig. 6. Thyroid glands of *Rana aurora draytoni* minus hypophysis (ventral view). Killed March 24 of succeeding year, 402 days old. Total length 56.7 mm., trunk length 20.9 mm., hind-leg length 1.68 mm. $\times 44$.

Fig. 7. Thyroid glands of *Rana aurora draytoni* minus hypophysis plus intermediate lobe of hypophysis (ventral view). Implant March 19, killed July 23. Total length 57.4 mm., trunk length 18.0 mm., hind-leg length 0.64 mm. $\times 44$.

Fig. 8. Thyroid glands of *Rana aurora draytoni* minus hypophysis plus posterior lobe of hypophysis (ventral view). Implant July 17, killed August 17. Total length 43.5 mm., trunk length 18.0 mm., hind-leg length 0.7 mm. $\times 44$.

Fig. 9. Thyroid glands of *Bufo boreas halophilus* minus hypophysis (ventral view). Killed January 24, almost one year old. Total length 40.5 mm., trunk length 15.0 mm., hind-leg length 2.16 mm. $\times 44$.

Fig. 10. Thyroid glands of *Bufo boreas halophilus* minus hypophysis plus anterior lobe of the hypophysis (ventral view). Implant October 4, died January 1. Total length 39.0 mm., trunk length 15.5 mm., hind-leg length 14.1 mm. $\times 44$.



STUDIES ON THE PERMEABILITY OF
LIVING CELLS

IX DOES METHYLENE BLUE ITSELF PENETRATE?

B1

MATILDA MOLDENHAUER BROOKS

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 6, pp. 79-92, 3 figures in text

Issued December 20, 1927

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

STUDIES ON THE PERMEABILITY OF LIVING CELLS

IX. DOES METHYLENE BLUE ITSELF PENETRATE?

BY

MATILDA MOLDENHAUER BROOKS

INTRODUCTION

In preliminary experiments (Brooks, 1926) on the penetration of methylene blue into living cells of *Valonia*, it was noted that this dye penetrated the sap at any pH value of the external solution from 5.4 to 9.0. Since the publication of this work, the writer has had an opportunity to extend her observations on the penetration of this dye.

It is the purpose of this paper to show that the amount of methylene blue found in the sap at equilibrium is apparently independent of the pH value of the external solution; that the rate of penetration of methylene blue is influenced by the temperature and pH of the external solution, and very probably also by light; and that methylene blue penetrates the sap of *Valonia* as such and not in the form of a lower homolog, such as trimethyl thionine.

METHODS

The same methods were used in this study as in the writer's previous work (1926) on *Valonia*. The methylene blue was from the same lot as that used in the former experiments. For presentation of this very pure sample, the writer is greatly indebted to Dr. W. M. Clark, formerly of the Hygienic Laboratory, United States Public Health Service, where it was made.

The dye was rubbed up in a small amount of distilled water and then added to sea water. In this way, the settling out of the dye, as subsequently reported by Irwin (1927), was avoided. The solutions of dye were made up each day just before using, in order to avoid chemical changes in the dye due to standing. The concentration used was 0.000047 M.

The plants were placed in buffered sea water containing methylene blue. The pH of the sea water containing the dye was adjusted at different values from 5.8 to 9.0 by the use of Clark's (1922) phosphate and borate buffers, the concentration of buffer salts being kept constant. At intervals of one-half, one, two, and three hours some plants were taken out of the solution, quickly rinsed in water, wiped dry on filter paper, and cut with a sharp piece of glass. The sap, which is under pressure, spurts out readily and is collected in a glass tube. The sap in this case is always blue and the addition of NaOII or H₂O₂ will not intensify the color. This shows that methylene blue penetrates in the oxidized form rather than in the reduced form. This seems to be due to the fact that the oxidation-reduction potential of this dye is so low at the pH value found in the cell that neither protoplasm nor sap is able to reduce it.

All necessary precautions were taken to keep the sap during its extraction free from contamination by the rest of the cell. This point is mentioned particularly, since it might readily be inferred from Irwin's (1927) criticism that she considers the results obtained by the present writer to be due to such contamination. The most satisfactory way to demonstrate that no contamination takes place is to compare the writer's methylene blue experiments with the experiments of the same writer on 2-6-dibromo phenol indophenol (Brooks, 1926). In the latter case the dye is always found in the sap in a colorless form, while the dye in the external solution and in the cell wall is blue. When, however, the sap is expressed in the same way as in the methylene blue experiments, the dye contained in the sap is always entirely in the colorless form. Had the sap become contaminated on its way out, with oxidized dye from the cell wall or an outside solution, it would not have remained colorless. Since the same methods and precautions were used with methylene blue as with indophenol it is safe to say that no contamination took place in these experiments as the dye was being extracted.

The concentration of dye in the sap was measured by comparison with standardized solutions of dye contained in tubes similar to those holding the sap. The dye used for these standards was made up fresh each day immediately before measurement as a routine procedure, because it was noted that the colors in the more dilute solutions changed on standing, so that they could not readily be matched. Concentrations less than 0.00001 M. seem to be unstable, while those of 0.000005 M. change color by the next day, and cannot be matched

with the dye in the sap. It is a well-known fact that methylene blue has a strong tendency to become adsorbed to glass surfaces. This may be responsible in part for the change in color observed in dilute solutions. Another explanation may be based upon Bernthsen's (1885) observation that alkaline solutions of methylene blue on standing become oxidized in air, forming lower homologs. Irwin (1927) notes that she had difficulty in matching the color of the sap with that of the standards at certain concentrations. No such difficulties are experienced when fresh standards are made preceding each experiment.

It is generally known that light influences in some way the permeability of living cells. For this reason, these experiments were done directly before a north window so that they received full diffuse daylight. That light affects the penetration of oxidation-reduction dyes into *Valonia* has been shown by the writer by experiments with 2-6-dibromo phenol indophenol, in which those plants which were kept in darkness showed very little penetration of dye into the vacuole as compared with those kept in light. Irwin (1927) reports that she was unable to find any dye in the sap at pH 5.5. This result was perhaps due to the fact that her experiments were done in very subdued light ("in an incubator with air holes"). It is difficult to make more definite suggestions since Irwin gives no data on concentrations of dye found in the sap, merely stating that "at pH 5.5 practically no dye penetrates while at 9.5 more enters the vacuole." These statements are not sufficiently quantitative to make possible further critical discussion of apparent discrepancies between Irwin's (1927) findings and the writer's.

It was important to observe whether or not the plants were irreversibly injured. When this happens, the plants cytolize sooner than the controls. In all these experiments some of the plants were taken from the solutions of dye at various intervals, transferred to sea water, and kept for observation as to time of survival. That these plants were not irreversibly injured has been proved many times by observing them as long as a month and noting that they lived as long as the controls which had not previously been in a solution of dye. It is not safe to consider them uninjured "if they were found to be living a day or so after transfer from the test solution to sea water" as Irwin does. The writer has often found that cells which show no injury in two or three days after being transferred from solutions of dye to pure sea water, will cytolize after that, while the

controls continue to live. Only by taking the precaution of making the prolonged observation here described, which is not arbitrary but based on experimental evidence, can one definitely ascertain whether or not a plant has been irreversably injured. In all the experiments by the writer on *Valonia* and *Nitella*, the test described above has always been used, and it was found in every case, except where specifically mentioned, that the plants experimented upon by the writer survived as long as the controls. Data not safeguarded by such a stringent criterion of injury or its absence, are subject to possible error.

RESULTS

EFFECTS OF TEMPERATURE AND pH

In the present experiments the penetration of methylene blue was studied at two temperatures, 22.5° C and 25° C. It was found that, at the lower temperature the rate of penetration of methylene blue varied with the pH, but at the higher temperature the rate of penetration so increased that at the end of one hour the concentration of dye found in the sap was practically the same at all pH values. This confirms the writer's previous observations (Brooks, 1927) which were based upon experiments done at 25° C and indicates that the penetration of dye into the sap reaches an equilibrium which is independent of pH value of the external solution.

At the lower temperature, however, penetration was retarded sufficiently to make more detailed observations possible. At this temperature it was found that the rate of penetration of methylene blue increased as the pH value of the external solution increased. After four or five hours, when the external pH values were low, the concentration of dye in the sap equaled the amount which had accumulated in the sap in a less time when the external pH was 9.0. Several successive readings after this seemed to indicate that an equilibrium had been reached before injury set in. This point is being investigated further, and will be reported on in detail in a later communication. At temperatures lower than 21° C the time necessary for penetration of the dye is so considerable that injury due to abnormal pH may set in before the dye penetrates.

IDENTITY OF THE PENETRATING DYE

The theory that methylene blue penetrates living cells as trimethyl thionine, a lower homolog, known also as methylene azure, has been promulgated by various writers. Among the earliest of these may be mentioned MacNeal (1906), Underhill and Closson (1905), Herter and Richards (1904), Scott and French (1924), and more recently Irwin (1927) with reference to *Valonia*. In cytological investigations, however, the fact that the nucleus is not stained blue by methylene blue is not proof that methylene blue does not penetrate. Methylene blue is colorless in its reduced form, and since the power of reduction is inherent in all living organisms, it may conceivably penetrate the cell and become reduced inside without visual evidence of any penetration.

In order to determine the identity of the dye which penetrates into the sap of living *Valonia*, spectrophotometric measurements were made. The absorption spectra of solutions in layers one centimeter thick contained in small cells made of optical glass were determined by the use of a Bausch and Lomb spectrophotometer. Each point on the graphs represents an average of from four to twelve determinations. The probable error of these measurements is less than one per cent of the mean.

Formánek and Grandmougin (1908) were the first to determine the absorption spectrum of methylene blue. Later, Holmes (1924) made a more detailed study of methylene blue at various dilutions. He showed that there is a shift in the wave length of maximum absorption from 660 m μ in aqueous solutions containing 25 parts of dye per million to 610 m μ in solutions containing 100 p.p.m.; and that there is a further shift to 600 m μ in solutions containing 300 p.p.m. In commenting on reasons for this change, he states, "the suggestion appears warranted that alteration in colloidal state may possibly constitute a factor of influence upon the molecular tautomerism." In this connection, it is interesting to note that Clark (1925), using methylene blue in solutions of some of the same concentrations that Holmes used, noted a remarkable variation of potential with concentration of dye, showing that as the concentration increased, the E_h decreased. Holmes also calls attention to the observation of Formánek and Grandmougin (1908) that a number of dyes exhibit various minor changes in the positions and relative intensities of their absorption bands occurring within a comparatively brief period

after the preparation of their solutions by dilution. These changes are apparently dilution effects which require an appreciable period of time for completion.

French (1926) found that as the concentration is varied from 2 to 10 p.p.m., there is a very appreciable effect on the value of the extinction coefficient.

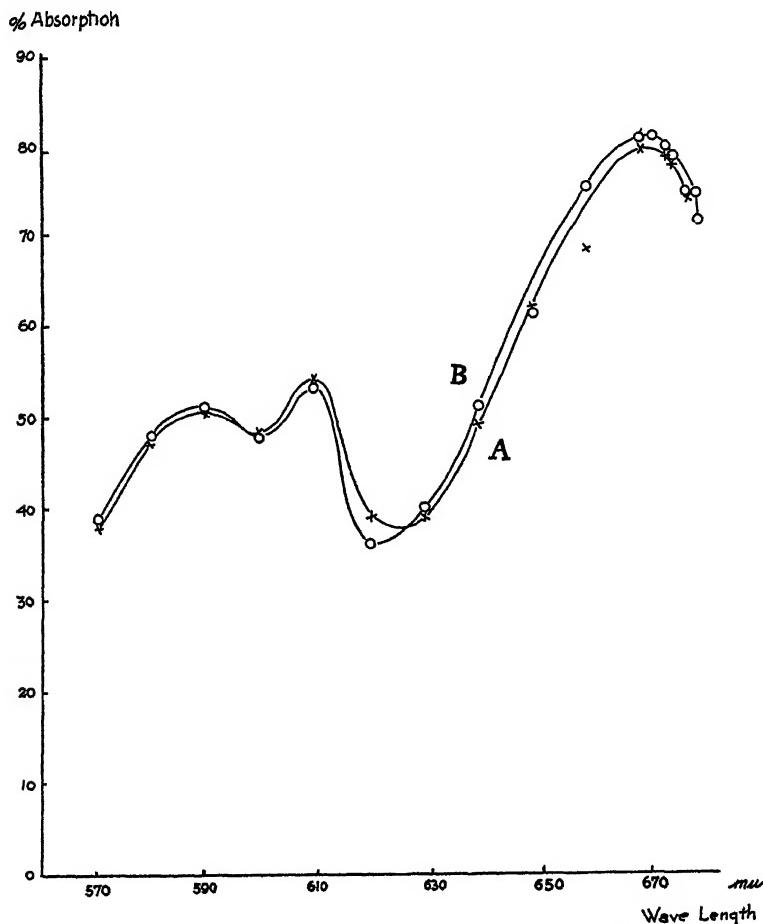


Fig. 1. Absorption spectrum of an aqueous solution of methylene blue, 0.000047 M. Curve A represents results obtained immediately after solutions have been made. Curve B represents results obtained 24 hours later. Abscissae, wave length; ordinates, per cent absorption.

The writer has also used the spectrophotometer to determine the identification of the dye. In these experiments the concentration of methylene blue in the outside solution was 0.000047 M. with a pH ranging from 5.8 to 9.0. Figure 1 shows two determinations of the

absorption of the methylene blue solution at pH 9.0. Curve A shows the absorption immediately after the solution was prepared; curve B, 24 hours later. They show that there is very little, if any change in the absorption spectrum of methylene blue after it has stood twenty-four hours in sea water at pH 9.0. The wave length of maximum absorption is at $667\text{ m}\mu$, with a secondary maximum at $610\text{ m}\mu$. This is essentially the absorption spectrum of methylene blue in aqueous

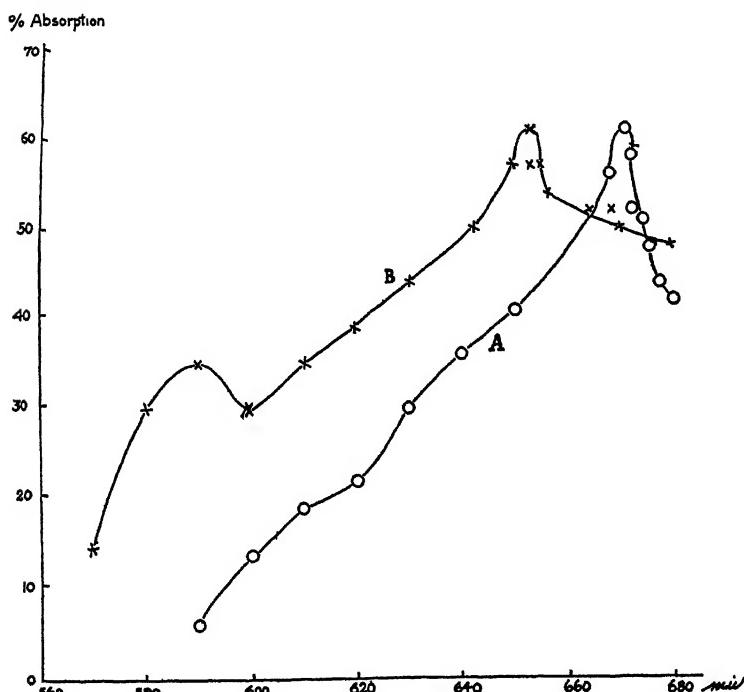


Fig. 2. Absorption spectrum of sap of plants which had been placed in methylene blue. Concentration of dye in sap is 0.000004 M. Curve A: absorption obtained immediately after sap had been expressed, showing absorption characteristic of methylene blue. Curve B: after sap had stood overnight, showing absorption characteristic of trimethyl thionine. Abscissae, wave lengths; ordinates, per cent absorption.

solutions of this concentration. Solutions whose pH was below 9.0 showed the same absorption maxima, and therefore need not be considered further.

The spectrophotometric study of the sap of plants which had been in solutions of methylene blue yielded important evidence: when sap of plants, which had been placed in solutions of methylene blue at any pH from 5.8 to 9.0 from one to three hours, was expressed and *immediately* analyzed by the spectrophotometer, it gave a wave

length of maximum absorption characteristic for methylene blue. When the sap was examined *after having stood for some hours* it give the absorption spectrum of trimethyl thionine. This shows definitely that methylene blue penetrates as such and that, after it is expressed, it gradually oxidizes in the presence of air and forms lower homologs, notably trimethyl thionine.

Figure 2 represents two sets of readings of the sap containing methylene blue. Curve A shows the absorption of sap immediately after it had been expressed, and curve B, after it had stood overnight. These curves are selected from a number of similar curves which show the same results. The plants had been in the solution of dye at pH 9.0 for 3½ hours at 21° C. The concentration of dye in the sap as determined by colorimetric readings was 0.000004 M. In curve A, the wave length of maximum absorption is 667 m μ with a slight secondary maximum at 610 m μ . This is the absorption spectrum for methylene blue in dilute solutions. In curve B, however, the maximum absorption is at 653 m μ with a secondary maximum at 590 m μ . This is the absorption spectrum for trimethyl thionine. Experiments at lower pH values of the external solution gave similar results.

In the experiments represented by figure 2 the dye found in the sap was very dilute. Figure 3 shows another set of readings in which the concentration of dye found in the sap was greater. The external solution was the same as in figures 1 and 2, but the temperature of this experiment was 22.5° C instead of 21° C, so that the concentration of the dye in the sap in two hours was 0.000015 M. when the external solution was of pH 9.0. The curves show three sets of readings taken at different times. Curve A, within 15 minutes after the sap was expressed; curve B, after three hours; curve C, after two days. They show a progressive shift in the region of secondary absorption toward that characteristic of the lower homologs of methylene blue as evidenced by the gradual flattening of the curve around 610 m μ and the subsequent rise around 590 m μ . The region of the primary maximum at 663 m μ has not materially changed. This seems to be in agreement with certain observations of French (1926), who says that oxidation products of methylene blue materially influence the secondary absorption maxima in the vicinity of 610 m μ . In several other experiments, the formation of even lower homologs was indicated by the rise in the curve in the vicinity of wave lengths shorter than 590 m μ .

To sum up, spectrophotometer studies showed that methylene blue dissolved in buffered sea water or distilled water at any pH from 5.8 to 9.0 gave characteristic absorption spectra of methylene blue in dilute solutions. There was no progressive shift in the location of the maxima for at least twenty-four hours.

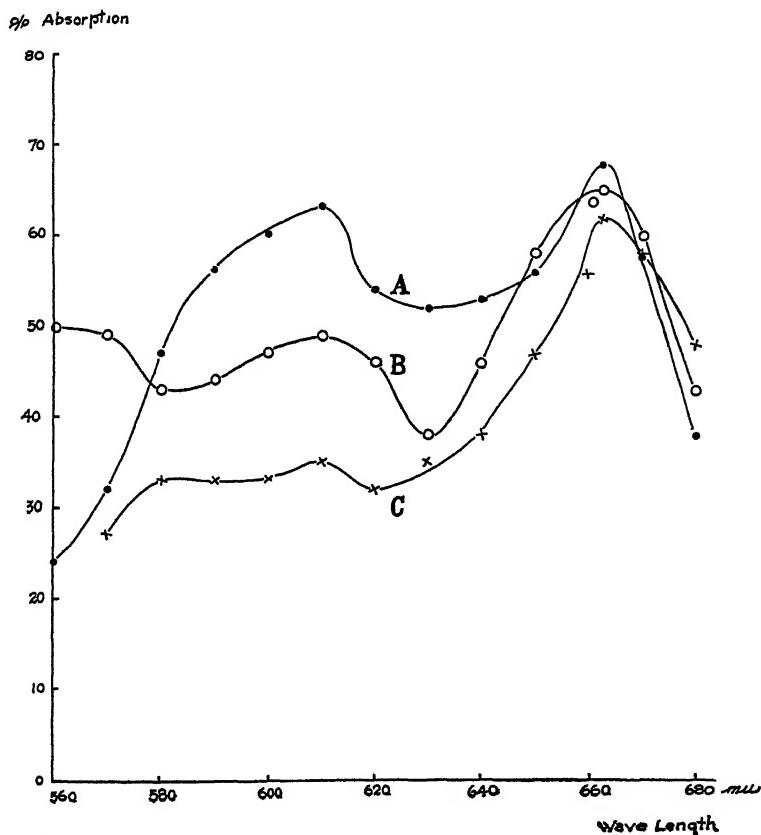


Fig. 3. Absorption spectrum of sap of plants which had been in solutions of methylene blue. Concentration of dye in sap is 0.000015 M. Curve A: readings taken immediately after the sap is expressed; curve B: readings taken several hours later; curve C: readings taken 48 hours later. Abscissae represent wave lengths; ordinates, per cent absorption.

The freshly expressed sap of *Valonia* cells which had been in solutions of methylene blue also gave the absorption maxima of methylene blue when it was examined immediately. When, however, it was allowed to stand for some hours, the wave lengths of maximum absorption and of secondary maximum absorption shifted to lower positions coinciding with the maxima for trimethyl thionine. This was best observed when the dye in the sap was dilute. When the methylene

blue became more concentrated in the sap owing to a higher temperature or to longer periods of exposure of the plants to dye solution, the primary wave length of maximum absorption remained the same but the position of the secondary maximum showed a progressive shift indicating the formation of lower homologs of methylene blue. This indicates that methylene blue as found in freshly expressed sap oxidizes on standing for some time and forms lower homologs. It is therefore necessary to make spectrophotometric determinations immediately after the sap has been extracted so that erroneous conclusions will not be reached as to the identification of the dye.

DISCUSSION

On the basis of the experiments here reported it seems impossible to avoid the conclusion that methylene blue penetrates as such into the sap, and not in the form of one of its lower homologs. Since Irwin states that when she exposed *Valonia* cells to methylene blue dissolved in sea water, she found the sap to give the absorption spectrum of trimethyl thionine only, one must also conclude that this homolog was formed by oxidation of methylene blue subsequent to extraction of the sap.

In this connection, Irwin (1927, p. 947) says, "Azure B found in the sap collected from the vacuole cannot be due to transformation of methylene blue into this dye after methylene blue has penetrated into the vacuole because no such transformation detectable by this method is found to take place within three hours after dissolving methylene blue in the sap of *Valonia*."

That methylene blue oxidizes in the presence of air has already been commented on in this paper. In these experiments on *Valonia* the change in oxidation-reduction potential of the expressed sap when exposed to air, as compared with intact sap, is very probably responsible for this oxidation of the dye. Only sap in which the methylene blue is quite dilute shows this change, perhaps because the change in E_h of the dye, which, as already mentioned, increases as the concentration of dye decreases, allows the dye to become oxidized in the expressed sap just as 2-6-dibromo phenol indophenol becomes oxidized in sap after exposure to air. In the latter case, this indophenol dye is always found in a reduced or colorless form in freshly expressed sap of plants placed for some time in the dye, and is oxidized

to a blue color when the sap is exposed to air. Furthermore, when this dye is added in small amounts to the sap *in vitro*, the dye never becomes reduced but remains blue, showing that sap in the cell is different from sap after it has been taken out of the cell and exposed to air. Irwin has neglected to note this fact: namely, that intact sap is not the same as sap *in vitro*; that sap containing dye as it penetrates through the protoplasm is not the same as sap to which dye has been added *in vitro*. Not only is the sap in the cell under conditions different from those *in vitro*, but also the action of the protoplasm on the dye as it penetrates must be considered. Therefore Irwin's reasoning in this connection is not justifiable, and her findings indicate only that her readings were not made immediately after the sap was expressed. They suggest that some time may have elapsed between her penetration experiments and the spectrophotometric measurements (which were made in Washington, D. C.).

The results are of further interest from the viewpoint of the penetration of electrolytes. Osterhout (1925) has shown that the concentrations of H₂S or CO₂ penetrating the sap of *Valonia* are proportional to the concentrations of their undissociated molecules in the outside solution. He concludes from this that only undissociated molecules penetrate living cells. Since methylene blue is a strong electrolyte it is completely dissociated at all pH values.

The dissociation of methylene blue has been studied by Clark (1925), who obtained no inflection of the curve in the alkaline region of the E_H-pH curve. From this he concludes that methylene blue is a very strong base and is completely dissociated at all pH values. He says (1925, p. 1166), "if the interpretation long accepted and confirmed by these studies is correct, then the dissociation of methylene blue chloride itself is such that no ordinary changes in pH can affect its degree." And yet, in spite of this complete dissociation, we find dye in the sap. How does this penetrate if there are no undissociated molecules in the external solution? While some substances show an agreement between the concentration of undissociated molecules in the external solution and the concentration of total substances penetrating, others do not show this agreement. In addition to the results here outlined on methylene blue, there are the experiments of the writer on the penetration of arsenic and arsenious acids into *Valonia*, and all the other experiments too numerous to list here, involving the penetration of strong electrolytes.

In order to account for the penetration of these strong electrolytes Osterhout (1926) has amended his former theory to agree with that proposed by Wilson (1923), who suggests that it is possible that two ions hitting the membrane simultaneously, momentarily associate and penetrate as such. It would therefore not be necessary to have undissociated molecules in the outside solution. Since this theory cannot be subjected to the test of direct experiment at this time, it does not seem profitable to discuss it at length.

It is evident, moreover, that this theory, which is based on the agreement in certain cases between the total concentration of substance found in the cell and the concentration of undissociated molecules in the external solution, is certainly not tenable in every case. Suffice it to say, that, until we have further data on several crucial points, conclusions as to the value of this hypothesis cannot be formulated.

The work of Michaelis (1925) on the permeability of the dried celloidin membrane suggests the possibility of accounting in another way involving ionization for the penetration of strong electrolytes and may make the Wilson-Osterhout theory unnecessary.

CONCLUSIONS

1. Methylene blue penetrates the living cell of *Valonia* at all pH values of the external solution.
2. Results indicate that the same equilibrium concentration of dye in the sap is eventually attained at all pH values of the external solution.
3. The rate of penetration of methylene blue varies with the pH of the external solution and with temperature.
4. Methylene blue penetrates the sap of *Valonia* as such and not as a lower homolog.
5. When the expressed sap is allowed to stand for some time, the transformation of methylene blue into its lower homologs can be demonstrated spectrophotometrically.

Grateful acknowledgments are made to the Bache Fund of the National Academy of Sciences for enabling the writer to procure *Valonia* in Bermuda and also to the Physics Department of Rutgers University for courtesies in the use of the spectrophotometer.

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BERKELEY, CALIFORNIA.

Transmitted October 27, 1927.

THE SEGMENTAL ARTERIES IN
SQUALUS SUCKLII

BY
ESTHER M. COLES

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 7, pp. 93-110, 10 figures in text

Issued February 3, 1928

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

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INTRODUCTION

A great deal of work has been done in recent years in the Zoological Laboratory of the University of California on the various organ systems of the elasmobranch fishes. For such studies the sharks and rays of the Pacific coast offer a particularly wide range of material, extending from the simplest and most generalized types to forms which show a high degree of specialization. Among the sharks, *Squalus sucklii*, is a relatively generalized type, and for that reason is valuable for a study of the arterial branches which are meristically arranged. This problem was suggested by and the work was done under the direction of Dr. J. Frank Daniel.

HISTORICAL

So far as I have been able to learn, little has been published on the segmental arteries and their branches in elasmobranchs. Hyrtl (1858) described briefly the paired branches of the dorsal aorta in *Torpedo narke* and *Raja clavata*. Sterzi (1904) studied in considerable detail the branches to the spinal cord in *Acanthias vulgaris*. I have not figured the arteries of the spinal cord since his article gives a description of these vessels, which is also accurate for *Squalus sucklui*. In studying the relation of the segmental arteries to the trunk myotome, the paper by Langelaan (1904) has been particularly helpful.

The segmental arteries are paired branches from the dorsal aorta which, for convenience, may be described in three groups. The first group includes the typical trunk segmentals arising from the dorsal aorta between the subclavian and the entrance of the aorta into the haemal canal. The second group consists of the segmentals anterior to the subclavian; and the third includes those of the caudal region.

SEGMENTAL ARTERIES OF TRUNK REGION

In the trunk region the branches of the segmentals supply blood to the renal organs, to the ventral, lateral, and dorsal musculature, and to the spinal cord. Before considering these branches in detail it is highly desirable to describe the trunk myotome with which these arteries are intimately associated.

A TRUNK MYOTOME

Figure 1 represents a typical myotome, bounded by an anterior and a posterior myoseptum, in the region of the first dorsal fin. The myotome is interrupted at two places (*ll.* and *ll'*, figs. 1 and 2) by septa of connective tissue, which descend and seem to divide the myotome into three parts, (1) an extensive dorsal part, (2) a short median segment, and (3) a ventral part. The first line of interruption (*ll.*) is at the lateral line. At this point, as Langelaan (1904) says, "the myotome appears to have been rolled in toward the axis of the body and reversed until it reaches the surface again." At the second line of interruption the folding is of such a nature that the ventral

part of the myotome overlaps the median segment. The myosepta beginning at the middorsal line (*ms.*, fig. 1) run posteriorly, almost parallel, and are separated only by a very thin lamella of muscle fibers. Their direction is then sharply reversed (V_1) so that they run anteriorly almost parallel to their former direction. Next they bend on an acute angle (V_2) and run backward. They then turn forward and downward (V_3) to the lateral line (*ll.*). Here the fold carries

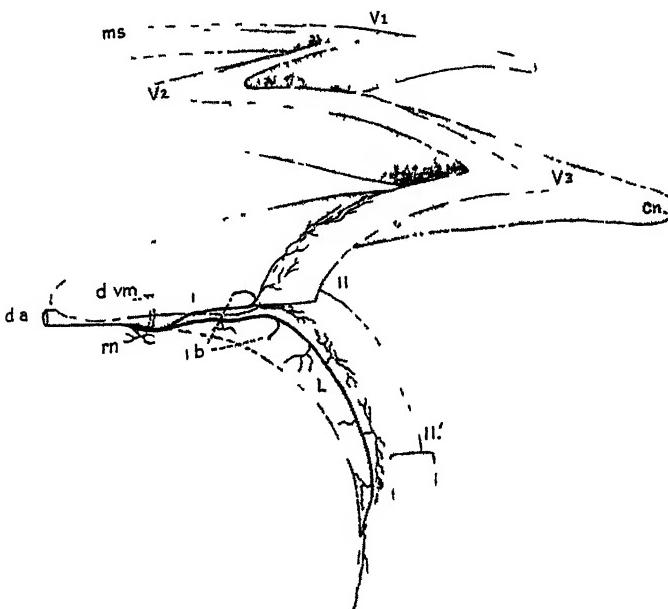


Fig. 1. Trunk myotome in region of first dorsal fin, *Squalus sucklii* ($\times 1$)
cn., extension of myosepta forming cone; *d.a.*, dorsal aorta; *d.v.m.w.*, dorsal vertebral muscle artery; *i.*, intercostal artery; *i.b.*, intercostal branches to preceding segments; *L.*, lateral bundle; *ll.*, lateral line fold; *ll'*, fold between lateral and ventral musculature; *ms.*, myoseptum; *rn.*, renal artery; V_1 , V_2 , V_3 , bends in myosepta.

them forward and inward almost to the rib processes. . The direction of the myosepta is then again reversed, and, attached to the rib processes, they run backward and outward to the surface. When the septa emerge from the fold they are displaced backward about a half-segment from the same myosepta above the fold. On this point the results of my investigations do not coincide with those published by Langelaan (1904), in which he states that the myosepta below the lateral line fold are cranially displaced one-half the breadth of a segment. In the lateral bundle (*L.*) the septa run parallel and in a straight line slightly posterior and downward to the second line of

folding (*ll'*). Here the fold carries them downward and inward to the peritoneum lining the body cavity. Their direction is then reversed and they fold back upward and posteriorly, emerging again at the surface in the ventral bundle about half a segment posterior to the same myosepta above the line of folding. The displacement at this second fold varies according to the location of the myosepta. Anterior to the region of the first dorsal fin it amounts to almost three-fourths of a segment. Posteriorly the displacement is gradually

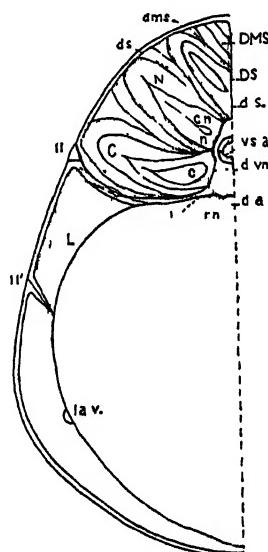


Fig. 2. Cross-section in trunk region, *Squalus sucklii* ($\times \frac{3}{4}$). *C.*, central muscle bundle; *c.*, central arteries; *cn.*, extension of myosepta forming cone; *d.a.*, dorsal aorta; *DMS*, dorsomedian septal muscle bundle; *dms.*, dorsomedian septal arteries; *DS*, dorsoseptal muscle bundle; *d.s.*, dorsal septum; *ds.*, dorsoseptal arteries; *d.vm.*, dorsal vertebromuscular artery; *l.*, intercostal artery; *L.*, lateral muscle bundle; *la.v.*, lateral abdominal vein; *ll.*, lateral line fold; *ll'*, fold between lateral and ventral musculature; *N.*, neural muscle bundle; *n.*, neural arteries; *rn.*, renal artery; *rs.a.*, vertebrospinal artery.

decreased until just anterior to the pelvic girdle, where the septa above and below the fold meet at about the same point on the surface.

The bends in the myosepta give a zigzag appearance to the external surface of the musculature. The septa, however, do not extend directly in, but at each of the V's they extend obliquely inward far posteriorly or anteriorly in the direction of the V. On each side of the V, moreover, they curve obliquely inward in a slightly dorsal direction on the one side and ventral on the other, forming a series of hollow cones which fit one within the other.

In a transverse section (fig. 2) the cones appear in their relation to one another. There are three rows of these above the lateral line, the central (C.), neural (N.), and dorsoseptal (DS.). In addition, there is a small dorsomedian septal (DMS.) bundle which does not form a complete cone, due to the fact that the myosepta at this V (V_1 , fig. 1) extend more directly inward. It appears merely as made up of narrow lamellae in juxtaposition. As is indicated in figure 2 (cn.) the most anterior and posterior extensions of the myosepta forming the cones, meet beneath the muscle fibers and give rise to narrow pointed pockets. These are not attached to the spinal column or to the other septa. The sections of the myosepta between the cones or pockets running anteriorly and those pointing posteriorly are attached either to the spinal column or to the dorsal septum (d.s., fig. 2) which extends from the column to the middorsal line. There are three lines of attachment of the myosepta above the lateral line. The sections of the septa between the central (C.) and neural (N.) bundles are attached to the spinal column along a line joining the ventral margins of the intercalary plates. Those between the neural (N.) and dorsoseptal (DS.) bundles are attached along the dorsal surface of the neural arch, in the section shown in figure 2. Sections taken posterior to the first dorsal fin would show this line of attachment gradually moving dorsally up the dorsal septum (d.s.). The most dorsal line of attachment is very near the top of the dorsal septum, between the dorsoseptal (DS.) and dorsomedian septal (DMS.) bundles. In addition to being attached to the column, each myoseptum is also loosely attached to the preceding one for a short distance out from the column by threads of connective tissue. This fact has misled Knauer (1910) into speaking of longitudinal septa between the muscle bundles.

Upon opening the abdominal cavity of a specimen of *Squalus sucklui* and removing the viscera and renal organs, we get a view of the segmental arteries of the trunk region which I have represented in figure 3. There are about thirty-three muscle segments between the subclavian and the entrance of the aorta into the haemal canal, but the segmental arteries supplying them are greatly reduced in number, from fifteen to eighteen leaving the dorsal aorta on each side (d.a., fig. 3). These are irregular in position and show considerable variation on the two sides and in different specimens. As a general rule the segmental on one side alternates with that on the other, but this does not always obtain. Thus, in a certain segment a vessel may arise on each side from the dorsal aorta, while in other segments segmental arteries may be entirely lacking.

BRANCHES OF THE SEGMENTAL ARTERIES

After leaving the dorsal aorta (*d.a.*, figs. 2 and 3) the segmental artery gives a small ventral branch, the renal (*rn.*), to the mesonephros or "kidney." Immediately afterward, a dorsal branch, the dorsal vertebromuscular artery (*d.vm.*, figs. 1, 2, 3, and 10), passes between the rib processes and up the side of the vertebra and dorsal septum (*d.s.*) to the middorsal line, giving a median branch to the spinal cord and lateral branches to the muscle bundles. The segmental itself is continued to the surface as the intercostal (*i.*), which runs outward along the edge of the ribs and ends in the lateral and ventral musculature. Figure 2 shows a segmental and the relation of its branches to the muscle bundles in a diagrammatic way. The reason is that the dorsal vertebromuscular artery curves anteriorly in its dorsal course, while its branches are directed somewhat posteriorly. Consequently, a single cross-section would not show the full length of one segmental and all of its branches.

In ventral view the "kidneys" appear as longitudinal bands lying along the roof of the body cavity on each side of the spinal column and extending from the base of the liver posteriorly throughout the entire length of the body cavity. In their posterior part they broaden out somewhat and become much thicker than they are anteriorly.

RENAL ARTERIES IN FEMALE

Figure 4 is a ventral view of the renal arteries to the right mesonephros or "kidney" of an adult female. Similar vessels supply the left side but they have not been drawn. In the female the first renal artery on each side (*rn.*, fig. 4) usually arises at the fifth segment posterior to the subclavian artery (*s.cl.*, fig. 3). In one specimen, in which the right subclavian was displaced one segment anteriorly, the first renal arose at the sixth segment. From the anterior tip of the "kidney" throughout its entire length a renal artery (*rn.*, fig. 4) is present to each trunk segment. Thus, there are usually twenty-nine renal arteries in the female. In rare cases I have found that the renals may not arise as branches from the segmentals, but may come directly from the dorsal aorta just posterior to the origin of the segmental. As a rule, wherever there is a segmental artery (*s.*, fig. 4), the renal (*rn.*) arises from it immediately after it leaves the dorsal aorta (*d.a.*). On other segments, however, in which no segmental is present, the renal arises directly from the dorsal aorta. The renals of the anterior part of the "kidney" of the female are very small and branch deep in the dorsal part of the tissue (fig. 5A₍₁₎).

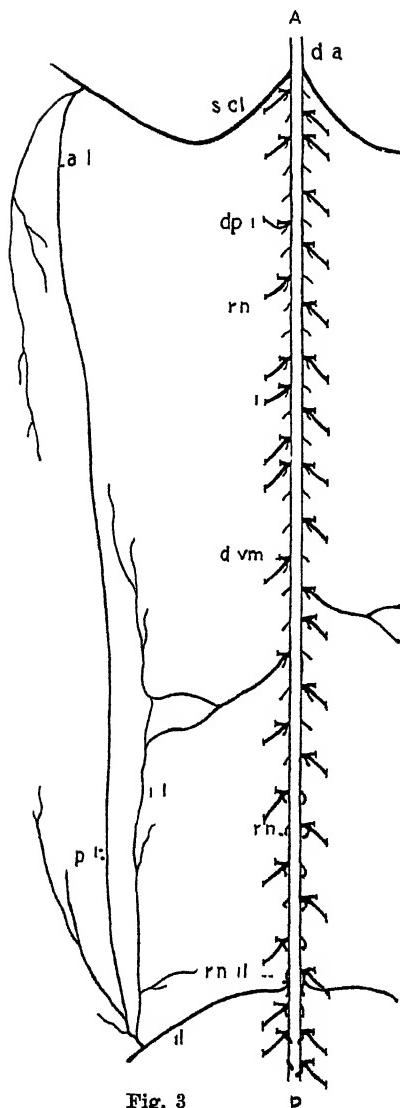


Fig. 3

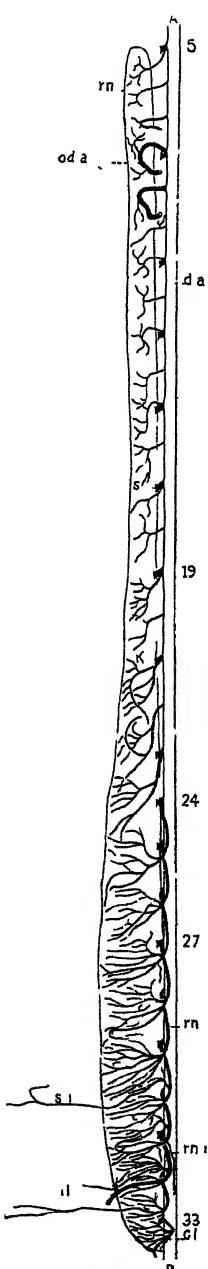


Fig. 4

Fig. 3. Ventral view of trunk segmentals, *Squalus sucklui* ($\times \frac{1}{8}$). A., anterior; a.l., anterior lateral artery; d.a., dorsal aorta; dp.i., displaced intercostal artery; d.v.m., dorsal vertebrromuscular artery; i., intercostal artery; il., iliac artery; i.l., inferior lateral artery; P., posterior; p.l., posterior lateral artery; rn., renal arteries; rn.il., renal-iliac trunk; s.cl., subclavian artery.

Fig. 4. Ventral view of renal arteries to right kidney of adult female, *Squalus sucklui* ($\times \frac{1}{2}$). A., anterior; c.a., cloacal artery; d.a., dorsal aorta; il., iliac artery; K., kidney; oda., oviducal arteries; P., posterior; rn., renal artery; rn.il., renal-iliac trunk; s., segmental trunk; s.i., superficial intercostal.

OVIDUCAL ARTERIES

Beginning at about the seventh or eighth segment two or three oviducal arteries (*od. a.*, fig. 4) arise on each side, either as branches from the segmentals proper or, where the latter are lacking, they arise directly from the aorta, apparently as modified renals. They pass out through the tissue of the mesonephros (*K.*, fig. 4) and curve backward to the median line after giving off small renal branches. In some cases there may be considerable anastomosing between them. Finally they leave the "kidney" and pass ventrally to branch in the walls of the oviduct and on the ovary.

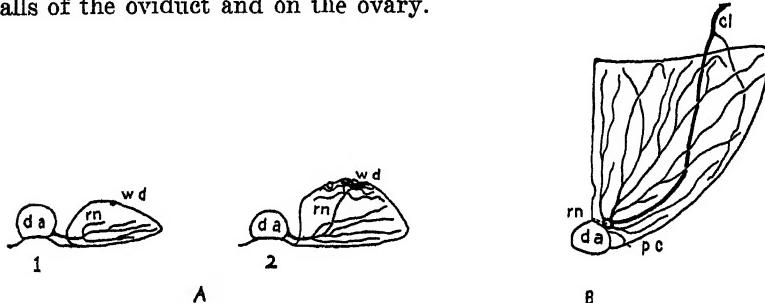


Fig. 5A. Cross-section through an anterior segment of the kidney, *Squalus sucklui*, (1) Female ($\times 4$), (2) Male ($\times 3$). B. Cross-section through the last segment of the female kidney, *Squalus sucklui* ($\times 3$). *cl.*, cloacal artery; *d.a.*, dorsal aorta; *p.c.*, postcardinal vein; *rn.*, renal artery; *w.d.*, Wolffian duct.

In the region of the nineteenth segment (19, fig. 4) the renals begin to take a posterior direction in the renal tissue, branching almost a segment posterior to their place of origin. They also become gradually enlarged and the branches are more and more numerous. At about the twenty-seventh segment (27, fig. 4) the "kidney" has become so much thicker that it presses up close against and over the aorta, meeting similar tissue from the other side. In this area the segments of the "kidney" are considerably longer than they are anteriorly. The renals now run posteriorly and ventrally along the inner margin of the "kidney." If the tissue is carefully removed, each renal may be traced as a single stem along the lateral surface of the aorta for more than the length of an entire segment. It crosses over the succeeding renal just posterior to the origin of the latter and then branches. This overlapping of the renal arteries begins at about the twenty-fourth segment (24, fig. 4) and continues back to the thirtieth or thirty-first. Here the segments shorten again and the trunks of the renal arteries also shorten so that the last two or three do not overlap.

In the posterior region, the postcardinal vein (*p.c.*, fig. 5*B*) runs in the roof of the "kidney" and close to the spinal column. Numerous veins of varying sizes run through the renal tissue and are accompanied by branches of the renal arteries. The arteries in general encircle these veins or sinuses and in cases they run outward for some distance to supply their walls. In this area the renal organ is very thick so that in addition to the lateral renal branches, other branches pass ventrally to supply deeper parts (fig. 5*B*).

SUPERFICIAL INTERCOSTALS

Small branches of the renals occasionally leave the kidney tissue and extend for some distance just under the lining of the abdominal cavity. These I have designated as superficial intercostals (*s.i.*, fig. 4). They are very irregular but usually two or three arise on each side in the posterior part of the trunk.

ILIAC ARTERIES

The iliac arteries (*il.*, figs. 3 and 4) arise at the posterior limit of the trunk. Their position varies from the thirtieth to the thirty-second segments and is not always the same on the two sides. Each iliac branches from a common renal-iliac trunk (*rn. il.*) which is identical with the other renals, except for its larger size. The large iliac branch passes through the mesonephros and at its margin gives off a small artery which divides in the trunk musculature. From there the iliac passes along the body wall just under the peritoneal lining to the region of the pelvic fin.

CLOACAL ARTERIES

In a similar manner a cloacal artery (*cl.*, figs. 4 and 5*B*) is given off on each side as a large branch from the last renal trunk. It passes through the tissue of the "kidney" to the posterior wall of the abdominal cavity. Here it runs ventrally under the peritoneum and branches in the posterior wall of the cloaca. It also gives off a few small branches to the dorsal musculature back of the cloaca.

RENAL ARTERIES IN MALE

The renal arteries in the male of *Squalus sucklui* differ but little from those of the female. The differences which do exist are due to the secondary relation which the mesonephrotic "kidney" bears to the genital system of the male. As a rule the first renal artery arises

at the third segment back of the subclavian artery making thirty-one renals on a side. The anterior part of the "kidney" is much larger than in the female and the blood supply is much greater. The renal first branches in the dorsal tissue as in the female, but one of the branches passes ventrally to the surface breaking up into a rosette of small vessels just beneath the coils of the Wolffian duct (*w.d.*, fig. 5*A*₍₂₎).

The blood supply to the posterior part of the male "kidney" is practically identical with that of the female. What has been said in regard to the superficial intercostals, the iliacs, and the cloacal arteries applies equally in the case of the male. Beginning near the twenty-fifth segment on each side and extending back as far as the thirtieth or thirty-first, branches from the renals pass directly down along the median margin of the "kidney" to the ventral surface of the seminal vesicle. Here they anastomose into a long arterial chain. The branch which leaves the iliac at the margin is a rather large vessel in the male. It sends small arteries into the musculature and back into the renal tissue, while the main branch passes ventrally along the surface to the seminal vesicle.

THE INTERCOSTAL ARTERIES

DORSAL AND VENTRAL INTERCOSTALS

As stated before, the trunk segmental is continued as the intercostal artery (*i.*, figs. 1-3) immediately after giving rise to the dorsal vertebrromuscular artery (*d. vm.*). The intercostal divides at once into a dorsal and a ventral branch (figs. 1 and 2). Both of these curve outward and upward to the surface along the lateral line fold (*ll.*). The dorsal branch follows the myoseptum which emerges above the fold, while the ventral branch follows the rib process, which is attached to the myoseptum on the ventral side of the fold. Figure 2 shows these branches of the intercostal in a diagrammatic way. Figure 1 shows them in their true relations. Since the branches of the intercostal run outward along the myosepta above and below the fold, both of them run posteriorly as well as outward and upward. The ventral branch, however, runs more posteriorly than the dorsal branch, due to the backward displacement of the septum below the fold. The dorsal branch, as a whole, has a spiral course, and on reaching the surface it follows the curve of the myoseptum dorsally and posteriorly almost to the first *V* above the lateral line (*V_s*).

It breaks up into numerous fine branches which run out into the muscle fibers on each side and into the connective tissue under the integument. The larger ventral branch, when near the surface, turns downward and posteriorly, following the myoseptum through the lateral bundle. Numerous fine branches pass to the surface, where they divide, running dorsally and ventrally along the outer margin of the septum surrounding the cutaneous vein (see Daniel, 1922, p. 226). Other branches from the ventral intercostal pass inward along the septum, while still others may supply the muscle fibers of the preceding myotome. Upon reaching the second fold (ll') the ventral branch of the intercostal follows the septum downward and inward to the bottom of the fold. Then it crosses over into the ventral bundle and follows the septum close to the lining of the coelom. It ends in the region of the lateral abdominal vein (*la.v.*, fig. 2).

As a rule each branch of the intercostal sends one or more branches to the preceding myoseptum (*i.b.*, fig. 1), in case the myoseptum lacks a segmental of its own. These branches divide and supply the segment in a way similar to that described above. Where there are two preceding segments which lack segmentals both of these are usually supplied by the same branches. Occasionally an intercostal passes as a whole to the preceding segment, leaving its own segment to be supplied with blood by anterior branches from the next intercostal (*dp. i.*, fig. 3). Only very rarely do branches supply a posterior segment, but they may occasionally do so where there are three segments between succeeding segmental arteries.

INFERIOR LATERAL ARTERY

One ventral intercostal on each side, varying in position from the nineteenth to the twenty-first segment, extends farther ventrally than the others and gives rise to an inferior lateral artery (*i.l.*, fig. 3). After crossing the second fold (ll' , fig. 1) it divides or forks into an anterior and a posterior branch, both of which are clearly visible just under the peritoneum. Shortly before reaching the lateral abdominal vein (*la.v.*, fig. 2) the anterior branch turns forward for several segments. The posterior branch runs backward to anastomose with the posterior part of the lateral artery (*p.l.*, fig. 3). The anterior and posterior branches may or may not be connected by a vessel extending across the outer edge of the V.

DORSAL VERTEBROMUSCULAR ARTERY AND ITS BRANCHES

After leaving the main trunk of the segmental, the dorsal vertebromuscular artery (*d.vm.*, fig. 2) passes between the rib processes around and up the side of the vertebra and dorsal septum to the middorsal line. In the region of the central column it gives off small central branches (*c.*, figs. 2 and 6) which run anteriorly and posteriorly along the cartilaginous wall. Where succeeding or preceding dorsal vertebromuscular arteries are lacking, these central branches may extend along the column for as much as two segments, often breaking up into a fine net. Frequently one of these small branches runs out into the musculature (*c.*, fig. 2).

Neural branches (*n.*, fig. 2) follow the myosepta which are attached to the column between the central and neural bundles. As stated previously, each myoseptum is loosely attached to the preceding one for a short distance out from the column by threads of connective tissue. The dorsal vertebromuscular artery usually gives off one or two main neural branches. Then as the myosepta separate and pass dorsally and ventrally, the vessels divide into dorsal and ventral branches similar to those of the intercostal artery. The dorsal branches follow the septa which pass to the neural muscle bundle (*N.*), while the ventral branches follow those of the central bundle (*C.*). Some of the neutrals extend to the outer surface, where they break up into fine branches under the integument. Figure 6 is a view of the dorsal vertebromuscular arteries (*d.vm.*) of the trunk region showing their central (*c.*) and neural (*n.*) branches.

A third group of vessels, which are not quite so numerous as are the neutrals, arises from the dorsal vertebromuscular artery between the neural (*N.*) and dorsoseptal (*DS.*) muscle bundles. These I have named the dorsoseptal arteries (*ds.*, figs. 2 and 7). They divide into dorsal and ventral branches which follow the septa out into the dorsoseptal and neural muscle bundles. In case the dorsal vertebromuscular artery divides after giving off the neural arteries, the dorsoseptals arise from each of the two branches, both of which usually continue on up the dorsal septum. The twenty-third dorsal vertebromuscular artery shown in figure 7 is an exception. Here the posterior branch does not continue. The twenty-first is an example of one which divides immediately after giving rise to the dorsoseptals. Very small vessels frequently pass into the musculature between the neutrals and the main dorsoseptals. These I have called superficial dorsoseptals (*s.ds.*, fig. 7).

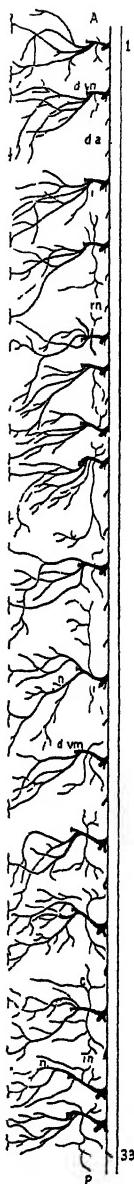


Fig. 6

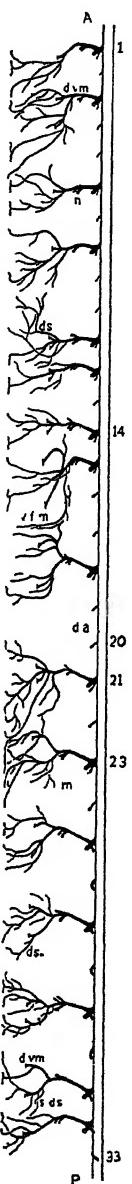


Fig. 7

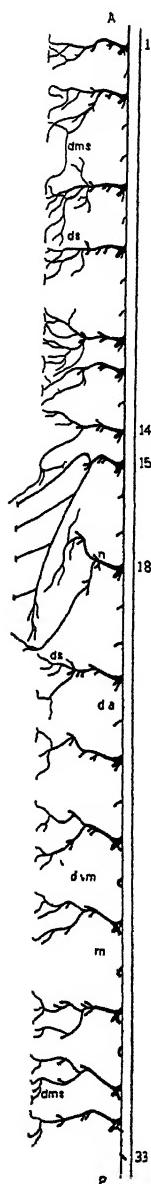


Fig. 8

Figs. 6-8. Side view of right dorsal vertebrromuscular arteries of trunk region, *Squalus sucklji* ($\times \frac{1}{2}$). Fig. 6. Central and neural branches. Fig. 7. Dorsoseptal branches. Fig. 8. Dorsomedian septal branches and specialized arteries to dorsal fin. A., anterior; c., central arteries; d.a., dorsal aorta; d.m.s., dorsomedian septal arteries; d.s., dorsoseptal arteries; d.v.m., dorsal vertebrromuscular artery; i., intercostal artery; n., neural arteries; P., posterior; r.n., renal arteries; s.d.s., superficial dorsoseptal arteries; v.f.m., ventral fin margin.

ARTERIES TO FIRST DORSAL FIN

From about the fourteenth to the twentieth segments, varying one segment anteriorly or posteriorly in different specimens, the dorsal vertebrromuscular arteries and their branches are greatly modified by the first dorsal fin. The point of origin of the dorsoseptal arteries is pushed down to the ventral margin of the fin cartilage, close to the dorsal surface of the spinal column. Fine branches follow along this margin anteriorly and posteriorly (*v.f.m.*, fig. 7). Figure 8 shows the dorsal vertebrromuscular arteries of this area continued as specialized vessels to the dorsal fin. The posterior branch of the artery at segment fourteen (14, fig. 8) follows the anterior margin of the fin cartilage. The posterior branch of the eighteenth artery (18, fig. 8) follows its posterior margin. The main blood supply to the fin itself, is furnished by the two branches of the fifteenth dorsal vertebrromuscular artery (15, fig. 8). The anterior branch runs almost as a straight vessel along the inner margin of the spine and out to the tip of the fin, while the posterior branch curves posteriorly along the side of the fin and sends several branches out along the fin rays.

The other dorsal vertebrromuscular arteries continue dorsally, often branching on the dorsal septum. Between the dorsoseptal and dorso-median septal bundles each branch usually gives off a small dorso-median septal vessel (*dms.*, fig. 8). This either runs directly to the surface along a myoseptum, or it may first extend anteriorly or posteriorly for some distance along the line of attachment of the myoseptum to the dorsal septum. The main trunks of the dorsal vertebrromuscular arteries continue to the middorsal line.

VERTEBROSPINAL ARTERIES

At the region of the neural arch each dorsal vertebrromuscular artery gives off a vertebrospinal branch (*vs.a.*, fig. 2) to supply the spinal cord. This passes in with the ventral root of the spinal nerve. Inside the neural canal it divides into two branches. The larger ventral branch, the *ramus ventralis*, is the direct continuation of the main artery. It passes between the ventral nerve roots, crosses the ventral surface of the cord, and runs into the large *arteria spinalis* which lies along the midventral line of the cord. The dorsal branch, the *ramus dorsalis*, is a slender artery, which runs dorsally along the lateral surface of the cord and divides into an anterior and a posterior branch. These branches anastomose with the corresponding branches

of the neighboring segments. Thus, by a fusion of these branches, a long *tractus arteriosus lateralis* is built up on each side of the cord, running along it near the bases of the dorsal nerve roots. Numerous fine branches, especially from the *tractus arteriosus lateralis*, press into the substance of the cord and branch in the gray matter. The vertebrospinal arteries and their branches are much more fully described by Sterzi (1904).

Usually each segmental artery has all of the above described branches. In nearly every specimen examined, however, I have found at least one segmental which was not continued by an intercostal artery, although its other branches were perfectly regular. In other cases I have found that the dorsal vertebromuscular artery ended with the neural branches. In some specimens the main trunk of almost every dorsal vertebromuscular artery continues as a single stem to the middorsal line. In others, practically every one divides into two main branches in the region of the neutrals or dorsoseptals, both of which continue to the middorsal line. The number and arrangement of the vessels in the various groups of arteries arising from the dorsal vertebromuscular arteries varies considerably, but the general plan which I have outlined is the same in all specimens of *Squalus sucklui*.

SEGMENTALS ANTERIOR TO SUBCLAVIAN ARTERY

The segmentals of the second group, or those anterior to the subclavian arteries, are smaller and are few in number. Usually there are three on each side and they may or may not be paired. The first two on each side arise from the paired dorsal aortae. On these segmentals renal branches are lacking, and the intercostals extending outward along the lateral line fold are much smaller vessels, seldom, if ever, reaching the surface and never bearing ventral branches. The dorsal vertebromuscular arteries give rise to the same groups of branches as in the trunk, though there are fewer vessels in each group. The central branches anastomose along the column forming a chain which extends from the first dorsal vertebromuscular artery back to the subclavian artery. One large branch arises from the first segmental on each side shortly after it leaves the aorta, and passes dorsally and anteriorly over the ear capsule. It crosses the path of the tenth cranial nerve and gives to it a small vessel; finally it branches on the ventral surface of the musculature covering the ear capsule.

SEGMENTALS OF CAUDAL REGION

The segmentals in the caudal region (fig. 9) differ somewhat from those of the other two groups described. They are smaller than the trunk segmentals, are regular—one to each body segment, and are paired. The intercostal (*i.*) is modified by an irregularity in the lateral line fold (*ll.*) which extends as far as the caudal fin. The fold no longer reaches the central column, and it gradually shortens and disappears. The displacement resulting from the fold becomes at the same time steadily smaller. The intercostal artery (*i.*) divides

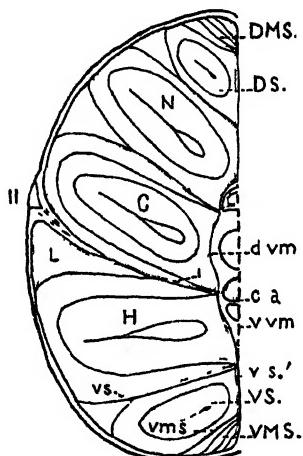


Fig. 9. Cross-section in caudal region anterior to second dorsal fin, *Squalus sucklii* ($\times 1$). *C.*, central muscle bundle; *c.a.*, caudal aorta; *DMS.*, dorsomedian septal muscle bundle; *DS.*, dorsoseptal muscle bundle; *d.vm.*, dorsal vertebromuscular artery; *H.*, haemal muscle bundle; *i.*, intercostal artery; *L.*, lateral muscle bundle; *ll.*, lateral line fold; *N.*, neural muscle bundle; *VMS.*, ventromedial septal muscle bundle; *v.m.s.*, ventromedian septal artery; *v.s.*, ventroseptal artery; *v.s.'*, ventral septum; *v.vm.*, ventral vertebromuscular artery.

farther out at the inner margin of the fold. The second fold does not extend posterior to the pelvic girdle. A line of V's or bends in the myosepta takes its place, separating the small lateral bundle (*L.*) from the ventral musculature, which consists of three muscle bundles, the haemal (*H.*), ventroseptal (*V.S.*), and ventromedian septal (*VMS.*) bundles. While still within the cartilage of the haemal arch the segmental gives off a large branch, the ventral vertebromuscular artery (*v.vm.*), which passes ventrally along the haemal arch and down the ventral septum (*v.s.'*). Between the haemal and ventroseptal bundles, or just ventral to the haemal arch, the ventroseptal branch

or branches (*vs.*) are given off. A small ventromedian septal artery (*vms.*) arises between the ventroseptal and ventromedian septal bundles. The ventral vertebromuscular artery continues to the midventral line. The dorsal vertebromuscular artery arises as in the trunk region, passes dorsally around the column and up the dorsal septum. It gives rise to essentially the same arterial groups, with the exception of the renal. There are fewer vessels in each group, however, only one or two as a rule being present.

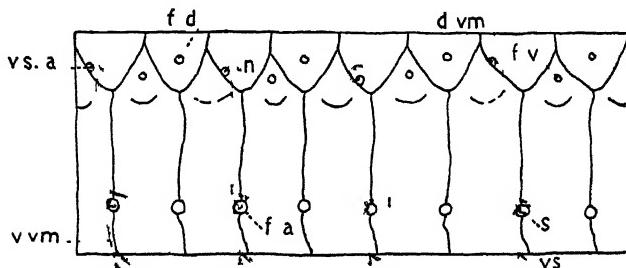


Fig. 10. Lateral view of column in caudal region, *Squalus sucklui* ($\times 1$). *d.vm.*, dorsal vertebromuscular artery; *f.a.*, foramen in haemal arch for segmental; *f.d.*, foramen for dorsal root nerve; *f.v.*, foramen for ventral root nerve; *i.*, intercostal artery; *n.*, neural artery; *s.*, segmental trunk; *vs.*, ventroseptal artery; *vs.a.*, vertebrospinal artery; *v.vm.*, ventral vertebromuscular artery.

The entrance of the vertebrospinal artery into the neural canal is modified by the diplospondylous condition of the column in the tail. The natural segments are longer than in the body region. To each of them two neural arches and two centra occur. The segmental passes out through a perforation in the haemal arch and its dorsal vertebromuscular branch runs up along the middle of the natural segment of the central column, bending slightly anteriorly in its course (*d.vm.*, fig. 10). Between the anterior basal and intercalary plates, which are not perforated by the nerve roots, the vertebrospinal artery (*vs. a.*) passes through a small foramen into the neural canal. Thus, in the tail region this artery does not enter with the ventral root of the spinal nerve, but has a canal of its own.

SUMMARY

A segmental artery in *Squalus sucklui* may be described as a derivative of the dorsal aorta, which, in the region of the trunk bears a small ventral renal branch to the "kidney," a dorsal branch, the dorsal vertebromuscular artery, which passes around and up the vertebra to the middorsal line, giving a median branch to the spinal cord and lateral branches to the muscle bundles; it is itself continued to the

surface as the intercostal. In the region anterior to the subclavian artery the segmental lacks the renal branch. The dorsal vertebral-muscular artery is present and the segmental itself is continued by a small intercostal. In the caudal area the segmental bears a large ventral branch, the ventral vertebral-muscular artery, which supplies the ventral musculature just as the dorsal vertebral-muscular artery supplies the dorsal muscle bundles. Both branches of the intercostal are present, but the ventral branch is much shorter than that of the trunk region.

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THE DERIVATIVES OF THE HYPOBRANCHIAL
ARTERIES IN *HEXANCHUS CORINUS*

BY

ANCEL B. KEYS

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY
Volume 31, No. 8, pp. 111-130, plates 5-8, 5 figures in text
Issued January 28, 1928

UNIVERSITY OF CALIFORNIA PRESS
BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS
LONDON, ENGLAND

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INTRODUCTION

In the vertebrates higher than the fishes the blood supply to the pectoral area is readily seen to be carried by the large subclavian artery which is derived from the dorsal aorta. The subclavian appears so early in the ontogeny of these vertebrates and assumes so uniformly important a position in all of these groups that the inference of its primitiveness is usually deduced with scant consideration of other possibilities. Anastomoses between the subclavian or its derivatives with vessels arising in the hypobranchial arterial system attract no comment so long as the subclavian, by its great size, remains indisputably the dominant artery. In the elasmobranch fishes, however, the hypobranchial arteries, by their increasingly large calibers, are seen to come more and more into prominence as we pass to the more generalized forms.

T. J. Parker (1886), working on *Mustelus antarticus*, found the subclavian to be directly connected with the "hypobranchial" artery, herein designated as the coracoid, near the point of origin of the brachial artery; but, judging from the relative size of the vessels, he concluded that the course of blood was from the subclavian to the

brachial and coracoid ("hypobranchial") arteries; in other words, that the coracoid arose from the subclavian. Daniel (1922 and 1926), investigating the same area in *Heptanchus maculatus*, found the subclavian to be a smaller vessel than the coracoid and seemingly second-

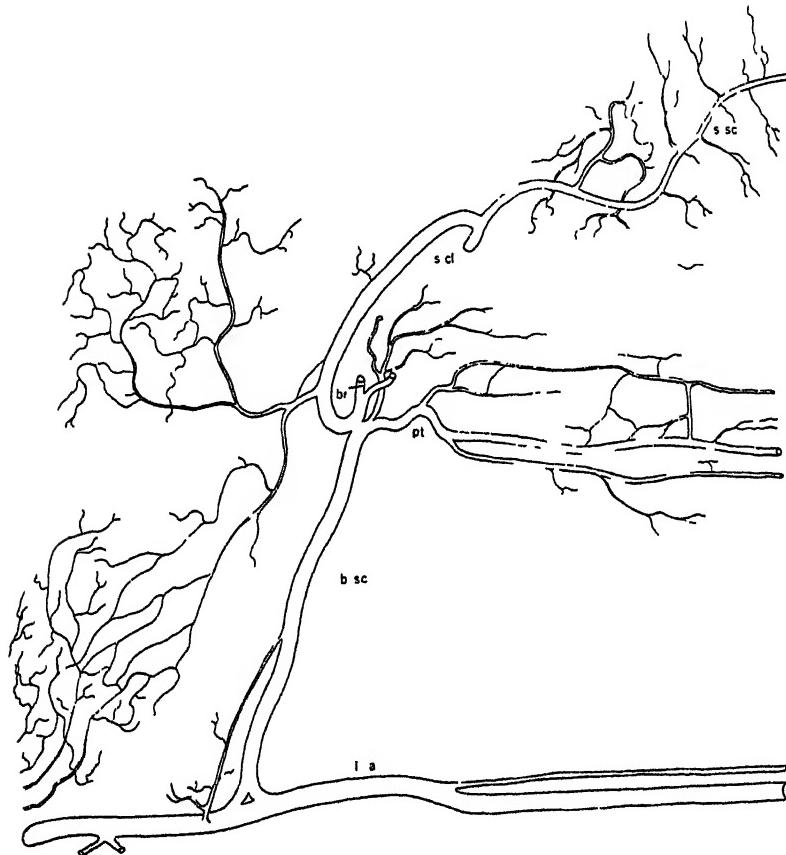


Fig. 1. Brachiosecapular artery and its relations, *Hexanchus corinus*, lateral view, left side.

b. sc., brachiosecapular artery; br., brachial artery; l. a., lateral (abdominal) artery; pt., pterygial artery; s. cl., subclavian artery; s. sc., subscapular artery.

ary in importance as a source of blood supply to the pectoral area. After careful examination of many specimens he reached the tentative conclusion that the subclavian arose as a common segmental of the dorsal aorta which, anastomosing with the coracoid, gradually usurped the function of the coracoid artery as the source of supply to the pectoral area.

Owing to lack of attention paid to this area by previous workers, perusal of the literature on the elasmobranchs has yielded little of

significance bearing on Daniel's theory. Even the common dogfish (*Acanthias*), although the subject of much investigation, has not to my knowledge been studied in this respect. It was assumed that significant conditions might be expected to appear in all of the more primitive elasmobranchs, particularly in *Hexanchus*, which like *Heptanchus* belongs to the Notidanids. In the spring of 1927, two specimens of *Hexanchus corinus* were received by the Department of Zoology of the University of California. These were injected and were turned over to me for a study of the arteries included in the hypobranchial and subclavian areas. I am greatly indebted to Dr. Daniel for advice and criticism during the course of the investigation.

METHOD AND GENERAL TOPOGRAPHY

The dissection was begun ventrally, and every artery, large and small, in the hypobranchial and pectoral areas was traced from its origin to its distal extremity, and all the larger vessels were measured and their calibers determined. The angles of the various arterial connections and the inclination of derivatives from the main arteries were studied in an effort to determine the direction of the flow of blood.

Dissection revealed a pectoral and hypobranchial musculature almost identical with that found in *Heptanchus maculatus* (Davidson, 1918). Removal of the ventral constrictors disclosed the coracoman-dibularis (*c. md.*, pl. 5) and the coracohyoideus muscles (*c. hy.*), between which the thyroid gland is situated. Portions of the ventral bundles dissected away showed the lateral (abdominal) arteries (*l. a.*) and veins (*l. a. v.*) coming into view just posterior to the girdle. Freeing the thyroid from its bed brought the thyroid arteries into view; and upon separating right and left coracohyoideus and coracoarcuales muscles, the ventral aorta (*v. a.*, pls. 6 and 7) and the complex hypobranchial arteries were disclosed. The course of the coracoid artery back of its origin and the origin of the lateral (abdominal) artery were revealed by removing the coracoarcuales muscles (*c. ar.*, pl. 5) and the coracoid cartilage (see pl. 8). The brachioscapular artery (*b. sc.*, pl. 8) and the origins of its brachial and metapterygial divisions were approached by dissection of the ventrolateral muscle bundle. The scapula was freed from its sheath of connective tissue, and the girdle with the pectoral fin attached was pulled to one side showing the

subscapular (*s. sc.*, fig. 1) and subclavian arteries (*s. cl.*) and all of their lateral connections. The body wall was opened at the point of emergence of the subclavian artery and the subclavian itself was traced to its origin from the aorta. The anterior (*a. dl.*, fig. 5) and posterior dorsolateral arteries (*p. dl.*) were demonstrated in the course of this part of the dissection.

In order to trace the coronary (*cr.*, fig. 4), pericardial (*pc.*), and posterior coronary (*p. cr.*, pl. 8) arteries, the pericardium was opened from the ventral side.

Of the two specimens of *Hexanchus* secured one was found to be in a poor state of preservation, especially in the more anterior portion of the hypobranchial region; the second specimen was well injected and perfectly preserved, and the detailed description refers particularly to it.

THE MEDIAN HYPOBRANCHIALS

The median hypobranchial artery (*m. hb.*, pl. 7) arises from the commissurals much as in *Heptanchus maculatus* (Daniel, 1922, fig. 149), although it is not so clearly defined as in the latter species. From the third to the sixth afferent arteries (*a. f.³⁻⁶*, pl. 7), the median hypobranchial is represented by a single vessel lying near the median line dorsal to the afferent arteries.

The median hypobranchial gives rise (1) to an azygos coracoid (*a. co.*, pl. 7) which is continued as the coracoid arteries, (2) to the coronary (*cr.*, fig. 4), and (3) to pericardial arteries (*pc.*, fig. 4).

AZYGOS CORACOID ARTERY

The azygos coracoid artery (*a. co.*, pl. 6 and especially pl. 7) in the specimen studied arises in the main as a large short vessel which passes ventrally and posteriorly from the left median hypobranchial between the third and fourth afferent arteries. On the right side the median hypobranchial was represented only by a network of smaller vessels connecting the commissurals; from this system of vessels on the right side several strong branches pass ventrally around the ventral aorta to join the unpaired (azygos) coracoid between the third and fourth afferents. In the region of the fourth, fifth, and sixth afferents an extremely complex system of small anastomosing

arteries connects the azygos coracoid with both left and right (?) median hypobranchials; this arterial plexus completely encircles the ventral aorta in this region. Numerous branches of this plexus unite in a thick short trunk which joins the azygos coracoid from the dorsal side at the region of the fifth afferent.

The unpaired or azygos coracoid artery (*a. co.*, pl. 7) continues posteroventrally and bifurcates at a point directly ventral to the origin of the sixth afferent (*af. 6*, pl. 7) as large right and left coracoid arteries (*co. a.*).

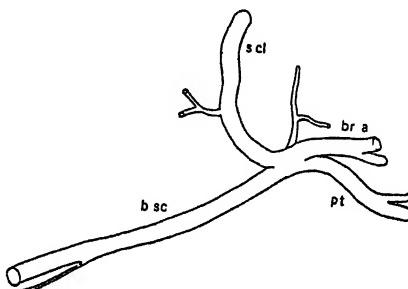


Fig. 2. Brachioseapular artery and its relations, *Hexanchus corinus*, anterior view (slightly ventral), left side. Lettering as in figure 1.

Anteriorly the azygos coracoid is continued beyond its point of main origin between the third and fourth afferents, as a fairly large median vessel passing along under the ventral aorta to a point just beyond its anterior extremity. Here it unites with both right and left commissurals (*cm. 1*, pl. 7) and with the unpaired anterior median hypobranchial artery which lies on the dorsal side of the ventral aorta.

In a posteroanterior direction this anterior prolongation of the azygos coracoid gives off, ventrally: (1) branches to the median portions of the first to the fourth coracobranchiales muscles and to the first to fourth interbranchial slips; (2) a strong branch to the coracohyoideus (pl. 5) and the coracomandibularis muscles; (3) a posterior thyroid artery, and (4) a large anterior thyroid artery (*a. th.*, pl. 7), this last-named artery arising at the point of union of the first commissurals, the anterior azygous coracoid and the dorsal unpaired median (hypobranchial) arteries. Dorsally the anterior prolongation of the azygos coracoid gives rise to two strong branches which pass respectively right and left around the ventral aorta between the second and third afferents; these branches unite dorsally forming a short dorsal median (hypobranchial) artery.

The thyroid arteries pass ventrally between the two coracohyoideus muscles, the anterior thyroid artery forming the main source of supply to the thyroid gland; whereas the posterior thyroid artery supplies only the posterior part of the gland, much of its blood going to the coracohyoideus and coracomandibularis muscles through various tiny branches.

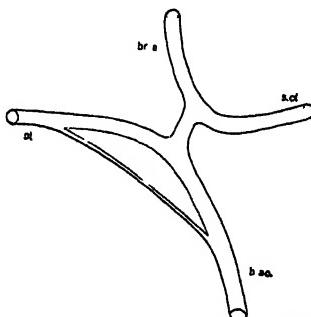


Fig. 3. Relations of brachioscapular artery, *Heranclus corinus*, anterolateral view, right side. Lettering as in figure 1.

CORACOID ARTERIES

The coracoid arteries (*co. a.*, pls. 6 and 8) continue backward from the azygos coracoid along the anterodorsal margins of the coracoid cartilages to form the lateral (abdominal) artery (*l. a.*, pl. 8). On the right side the coracoid artery passes under the cartilage and gives rise to the right lateral (abdominal) without bifurcating.

The posterior coronary (*p. cr.*, pl. 8) arises as a strong branch from the coracoid artery at the point where the subclavian vein (*s. cl. v.*, pl. 8) as a continuation of the lateral abdominal vein (*l. a. v.*) enters the sinus venosus. It then forms a loop and passes toward the median line into the pericardial wall. From the outer angle of the loop thus formed a complex system of small arteries arises which supply the posterior coracobranchial and ventral constrictor muscles. In this plexus a fairly large single trunk may be distinguished which bifurcates just ventral to the duct of Cuvier, one branch going to the median wall of the duct, the second and larger branch ramifying in its posterior wall. The latter branch from the posterior coronary artery sends out numerous small twigs to the posterior cardinal sinus, to the dorsal wall of the sinus venosus, and to the posterior portion of the pericardial wall. Several anastomoses between posterior coronary branches and the postbranchial plexus, associated with the subclavian artery (pl. 8 and fig. 1) are plainly visible.

THE BRACHIOSCAPULAR ARTERY

The term employed here, brachioscapular artery (*b. sc.*, fig. 1) is more completely applicable to the vein accompanying this artery than to the artery itself in that in the Notidanids the vein is composed of a brachial and a scapular (subscapular) division. The artery supplies some of the blood of the brachial artery (*br. a.*), but the subscapular

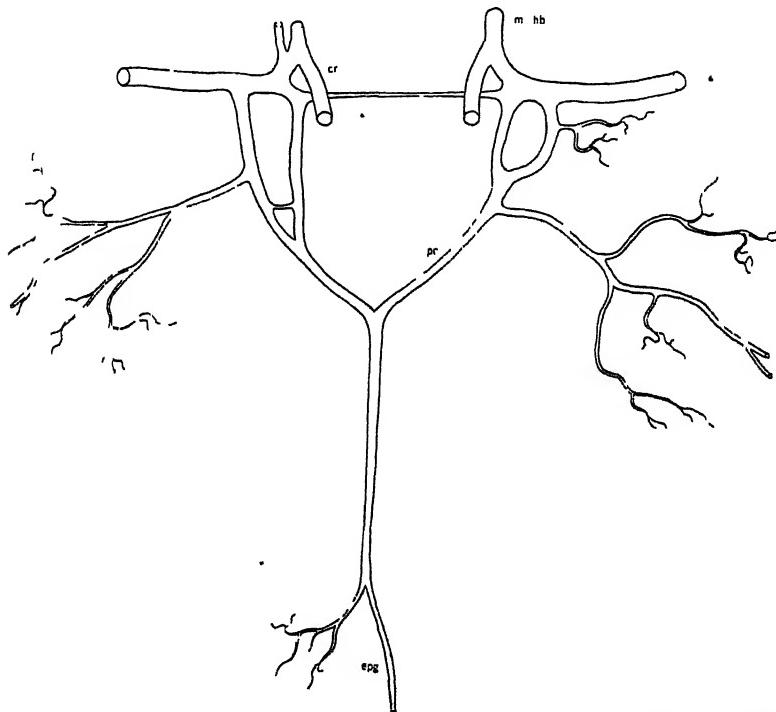


Fig. 4. Arteries derivative of the median hypobranchials, *Hexanchus corinus*, ventral view.

cm., sixth commissural artery; *or.*, coronary artery to heart; *epg.*, epigastric; *m. hb.*, median hypobranchial.

artery (*s. sc.*) is in reality a branch from the subclavian artery (*s. cl.*, fig. 1), while the segment from the brachial to the subscapular is the terminal part of the subclavian artery. The brachioscapular artery in *Hexanchus* is an unusually long segment due to the fact that in this species the lateral (abdominal) artery is relatively near the midventral line.

The brachioscapular artery (*b. sc.*, pl. 8 and fig. 1) arises from the lateral (abdominal) artery just posterior to the girdle. In plate 8 two

trunks of equal size are seen coming from the lateral (abdominal) artery (*l. a.*). These unite almost immediately to pass laterally and dorsally toward the pectoral fin. On the left side a third vessel of much smaller caliber arises from the lateral abdominal and passes laterally to unite with the brachioscapular at the point where it emerges from the lateroventral bundle. Just before reaching the pectoral girdle, at the foramen of the girdle, the brachioscapular artery (*b. sc.*) joins the subclavian (*s. cl.*, fig. 1) and from this union brachial (*br. a.*) and pterygial (*pt.*) arteries take origin. The brachial passes into the foramen of the girdle and the pterygial runs as an apparent continuation of the brachioscapular along the median side of the pectoral fin breaking up into a number of branches to the ventral radial muscles of the fin.

SUBCLAVIAN ARTERY

The subclavian artery (*s. cl.*, fig. 5) takes its origin from the dorsal aorta at the same angle and in apparently the same manner as the common segmentals. Its course is determined by the shape of the myotome in which it arises just as the course of the segmentals is determined. Following the course of the subclavian from the aorta (fig. 5) it is seen to be a fairly large vessel devoid of branches until about halfway to the lateral body wall where it gives rise to a short stout vessel, the common dorsolateral artery. The dorsolateral passes anteriorly for a short distance and then divides into the anterior dorsolateral (*a. dl.*, fig. 5) and the posterior dorsolateral (*p. dl.*) arteries. Still following the subclavian away from its origin it is noted that it increases in size slightly until it leaves the body wall. At this point it appears to bifurcate into (1) a smaller subscapular artery (*s. sc.*, figs. 1 and 5) which passes posteriorly and dorsally, branching profusely along the course of the scapula, and (2) the larger continuing subclavian, the caliber of which is somewhat greater than the more proximal part of the subclavian. This larger division continues ventrally and anteriorly until very close to the foramen of the girdle, where it turns sharply and passes through the foramen as the brachial artery (*br.*, fig. 1). Just as it turns sharply it comes in contact with and joins the brachioscapular artery. Before turning to form this union, however, the subclavian gives rise to a vessel which passes anteriorly and breaks up into the postbranchial plexus previously described (fig. 1; also pl. 8), between the last cleft and the girdle.

In the specimen dissected the union from which the pterygial and brachial arteries originate was studied with great care. On the left side (figs. 1 and 2) it appeared as if two separate vessels, a subclavian-brachial and a brachioscapular-pterygial had fused at a single point. On the right side (fig. 3) the place of fusion is drawn out into a special vessel. To this point we shall return later.

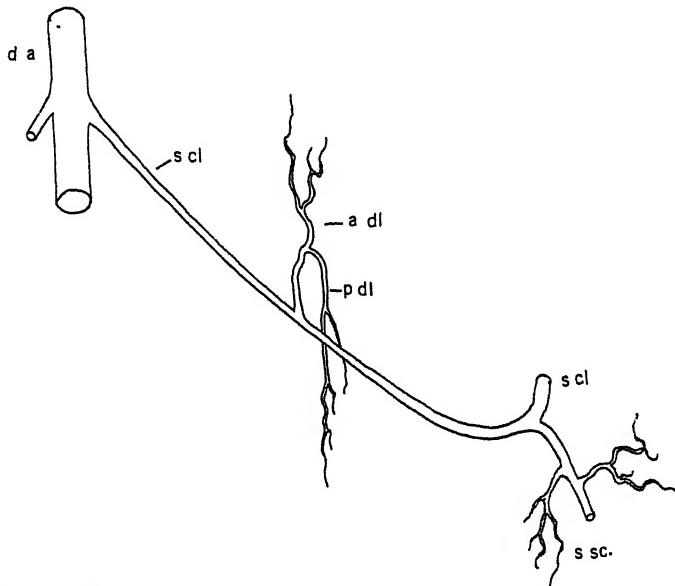


Fig. 5. Diagram of subclavian artery, *Heranchnus corinus*, ventral view. *a. dl.*, anterior dorsolateral artery; *d. a.*, dorsal aorta; *p. dl.*, posterior dorsolateral artery; *s. cl.*, subclavian artery; *s. sc.*, subscapular artery.

ARTERIAL SUPPLY TO THE HEART

The pericardial and coronary arteries (*pc.* and *cr.*, fig. 4) arise from the median hypobranchials (*m. hb.*) and the sixth commissurals (*cm.⁶*) in much the same way as they do in *Heptanchus* (see Daniel, 1922, figs. 149, 150). Both sides of the hypobranchial system, however, contribute to the formation of the pericardial which passes posteriorly along the dorsal roof of the pericardium giving off branches to the walls and continuing on to the digestive tract as the small epigastric artery (*epg.*).

The posterior coronary has already been described with the coccoid arteries. It is a fairly large vessel which rapidly dwindles in size as it branches over the posterior wall of the pericardium. Several very small twigs from it continue to the auricle.

EVIDENCE INDICATING THE COURSE OF BLOOD TO THE PECTORAL AREA IN *HEXANCHIUS*

In the specimen studied the brachioscapular artery (fig. 1 and pl. 8) was of a larger caliber throughout its entire length than the greatest caliber of the subclavian at any point; furthermore, the subclavian artery was found to be smaller close to its origin from the aorta than it was at a point near its union with the brachioscapular (fig. 5). An examination of the direction of branches from these two vessels showed that all branches of the brachioscapular artery were directed laterally at their origins, whereas the common dorsolateral artery, arising from the subclavian, at its origin was directed slightly back towards the aorta. No other branches of the subclavian were found between the lateral body wall and the aorta. In *Hexanchus* the pterygial artery (pt., fig. 1 and pl. 8) appears to be supplied wholly by the brachioscapular trunk, and it is certain that there is an appreciable flow of hypobrachial blood into the brachial artery. The effect on the subclavian of the greater pressure and volume of the blood carried by the brachioscapular is noted as close to the origin of the subclavian as the dorsolateral artery, where it influences the angle of departure of the common dorsolateral artery.

DISCUSSION

In *Heranclus* the brachioscapular artery, arising from a fusion of segmentally arranged arteries from the lateral (abdominal) artery forms the most important source of blood to the pectoral area. The subclavian arises as a segmental artery from the dorsal aorta and doubtless originally furnished a blood supply only to the more dorsal and lateral body regions. Its union with the brachioscapular and the pterygial arteries was formed secondarily; and this union still persists in *Hexanchus*.

It would appear, therefore, that the subclavian artery, as constituted in all of the higher vertebrates, is not the most primitive condition. Its evolution is readily understandable in the light of the Balfour-Thacher fin-fold theory. Roughly we may sum up the probable sequence as follows: There were two primitive types of vessels, the dorsal aorta and the lateral (abdominal) arteries which originally furnished the blood supply to the trunk. The dorsal aorta, in addition

to supplying the viscera, supplied the dorsal and lateral body walls by means of laterally directed segmentals (intercostals). The distal extremities of these segmentals from the dorsal aorta either directly or by fusion with branches from the lateral (abdominal) artery came to supply the dorsal and lateral portions of the fin-fold, while the branches from the lateral (abdominal) supplied the ventral and median areas. With the gradual specialization of the fin-fold into pectoral and pelvic fins the segmentally arranged arteries involved in these areas developed in proportion to meet the demand for increased blood supply. The two sources of supply persist as such in *Hexanchus*. With the further development and constriction of the fin at its union with the body, anastomosis between the brachioscapular artery and the subclavian artery was well developed, but the subclavian usurped the function of the brachioscapular artery, giving us the ordinary condition in the higher vertebrates where the subclavian forms the sole supply to the pectoral or shoulder area.

In *Hexanchus* we have a stage where the union of the subclavian-brachial and brachioscapular-pterygial stems still persists. The two sides (figs. 2 and 3) may well be considered as representing two stages in the loss of this union. If the two stems were separated still farther than is shown on the right side of *Hexanchus* (fig. 3) it is evident that this union would break and that the subclavian artery would become the only source of blood through the brachial artery to the pectoral area, as it is in all higher vertebrates.

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Transmitted October 4, 1927.

EXPLANATION OF PLATES

PLATE 5

Hypobranchial arteries, *Hexanchus cornutus*, ventral view. $\times \frac{1}{3} +$. *c. ar.*, coracoaculeus muscle; *c. hy.*, coracohyoideus muscle; *c. md.*, anterior portion of coracomandibular muscle; *hy. af.*, hyoidean afferent; *l. a.*, lateral (abdominal) artery; *l. a. v.*, lateral abdominal vein.

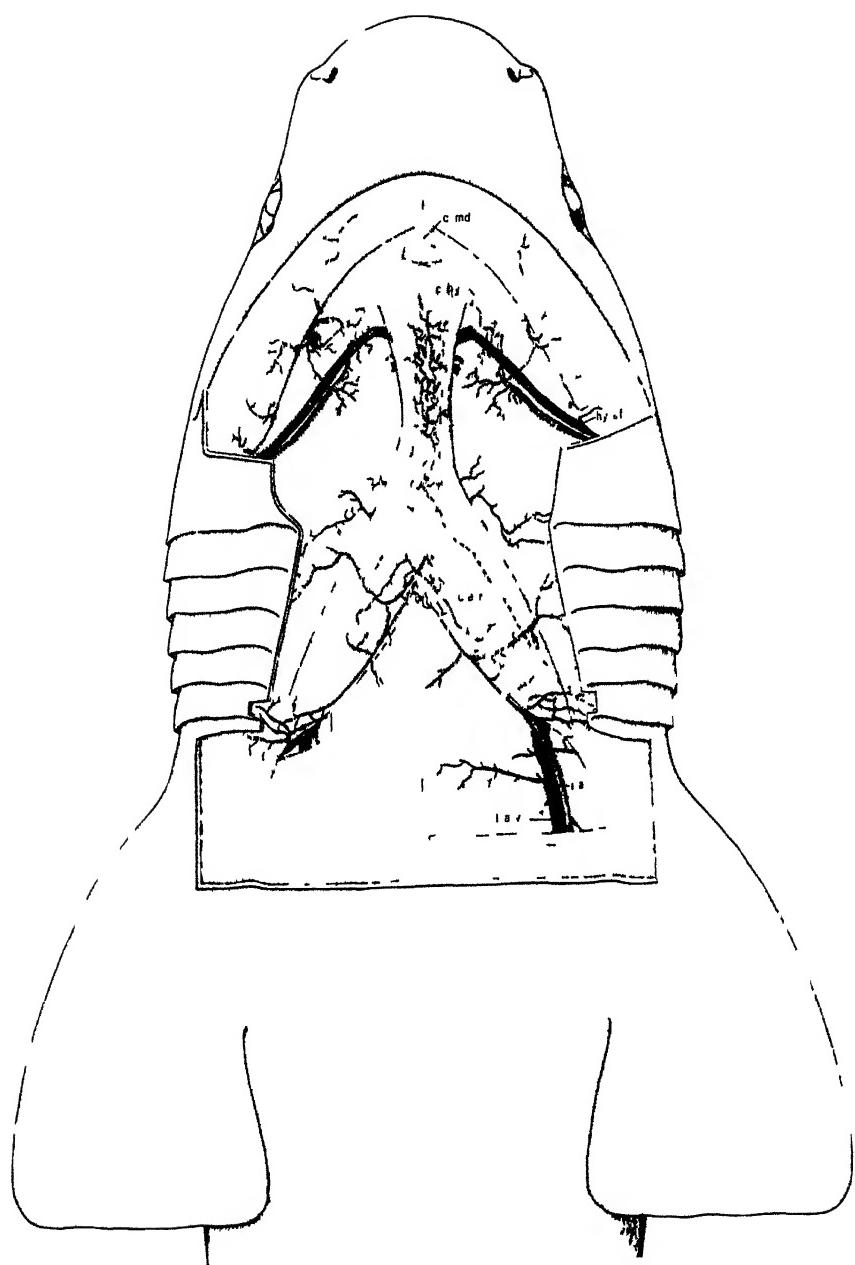


PLATE 6

Hypobranchial arteries, *Hemichasmus cornutus*, ventral view. $\times \frac{1}{4}$. *a.co*, azygos coeliac artery; *af.3*, third afferent artery; *co a*, coeliac artery; *l a*, lateral (abdominal) artery; *l a v.*, lateral abdominal vein; *v.a*, ventral aorta.

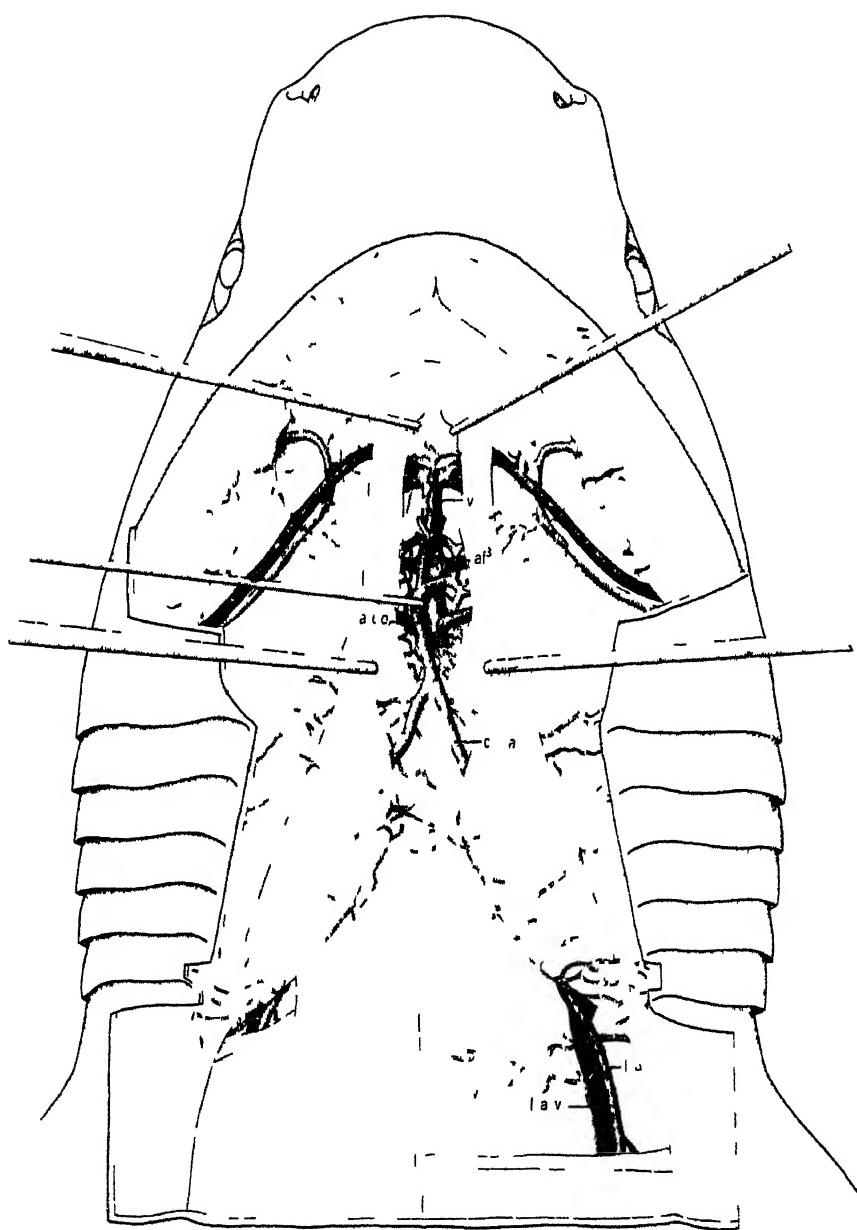


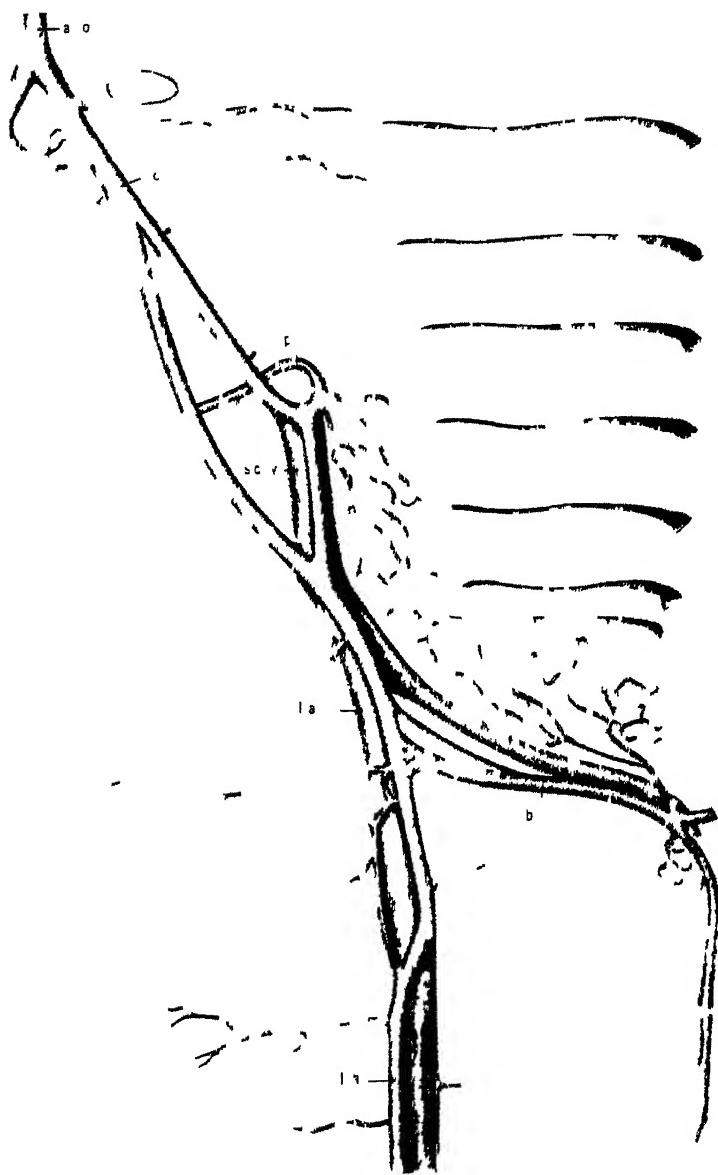
PLATE 7

Origin and relations of azygos coracoid, *Hexanchus cornutus*, ventral view. $\times 2.5$.
af. and *af.'*, third and sixth afferent arteries; *a. co.*, azygos coracoid artery; *a. th.*, anterior thyroid artery; *cm.*¹, first commissural artery; *co. a.*, coracoid artery; *m. hb.*, median hypobranchial artery; *v. a.*, ventral aorta.



PLATE 8

Relations of coracoid to lateral (abdominal) artery, *Hexanchus cornutus*, ventral view $\times \frac{1}{4}$
a co, azygous coracoid artery, b sc, brachioscapular artery,
co a, coracoid artery, l a, lateral (abdominal) artery, l a v, lateral abdominal
vein, p c, posterior coronary artery, s cl v, subclavian vein



LENGTH OF LIFE OF DROSOPHILA
MELANOGASTER UNDER ASEPTIC
CONDITIONS

BY

HELEN McDONALD STEINFELD

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 9, pp. 131-178, 8 figures in text

Issued February 24, 1928

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

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INTRODUCTION

The present investigation was planned to test the influence of certain selective agencies on length of life. The fruit fly, *Drosophila melanogaster*, was decided upon as the experimental animal, and the studies were limited to observations on the duration of life under aseptic and control conditions. The same strains of inbred flies were used for both the aseptic flies and the controls, and other environmental conditions were kept as nearly constant as possible. It was planned to use not fewer than five hundred flies of each of six strains in each part of the experiment.

A first set of controls was started in August, 1926, and eggs from these flies were sterilized to secure the starts for the six aseptic lines. As a matter of fact several generations of aseptic flies were raised before sufficient numbers were secured for all parts of the experiment. The first aseptic flies to hatch out were transferred to control duration-of-life bottles and run on Pearl's synthetic medium in order to observe what influence, if any, the aseptic larval and pupal period had upon the length of life of the adults. This is called in the records, Experiment I.

While both the first set of controls and Experiment I were running, Experiment II was started with the next groups of aseptic flies. These were transferred to aseptic duration-of-life bottles, fifty to a bottle, containing the standard banana agar medium, but no yeast. This, of course, is an aseptic medium with deficient food conditions, since bananas are low in protein and contain little Vitamin B, as is shown by the fact that the female flies soon cease egg-laying, and very few, if any, flies mature. Baumberger (1919) believed the inadequacy of bananas as a food for *Drosophila* to be quantitative rather than qualitative, since an occasional small pupa is formed when the food is in a concentrated form, as when somewhat dried out. The advantage of using this medium, from the standpoint of the experimenter, is the saving of labor, since fewer transfers are necessary to prevent the offspring from becoming mixed with the experimental flies.

Experiment III consisted of keeping aseptic flies on banana agar with 10 per cent yeast throughout their entire life. This necessitated transferring the imagos to fresh media every eighth to tenth day (depending on the type) to prevent mingling with the next genera-

tion. Five to eight transfers were required, and the percentage of bottles lost by the accidental introduction of microorganisms was correspondingly great. Therefore another scheme was tried, namely, isolation of the males and females in the duration-of-life bottles. This was successful in cutting down the number of transfers necessary, but since no larvae were present to liquefy the medium, it tended to become dry, and as it shrank away from the sides of the bottles many flies met accidental death by being caught in this region.

A second set of controls was started while Experiment III was in progress. Aseptic flies were put into ordinary breeding bottles and used as parents for these controls. This was planned to test what changes, if any, had taken place in the hereditary composition of the flies during the six to eight generations they had lived aseptically, and also to register whatever change there might have been with respect to the season, since the first set was started in August, and this second set in December. Obviously, all the experimental flies and controls could not be run at the same time, and it was therefore hoped that the average of the two sets of controls would give a very fair indication of the length of life of these six strains under non-sterile conditions.

The detailed data upon which the tables have been based are embodied in thirty-six typewritten tables which are on file in the Library of the University of California. These are referred to in the text by numbers 21-57. Photostat copies of these can be furnished by the Library at a moderate cost.

ACKNOWLEDGMENTS

This work was carried on under the direction of Dr. S. J. Holmes, whom the writer wishes to thank for criticisms and suggestions, especially as to the theoretical import of the problem.

Appreciation is expressed to the Department of Genetics of the University of California for supplying the six strains of *Drosophila melanogaster* used in the experiment, and for caring for my stock cultures during May and June, 1926. The author is also indebted to Miss Jean Moir, who made the bacteriological tests as to the asepticity of the duration-of-life bottles.

HISTORICAL REVIEW

ANIMAL LIFE UNDER ASEPTIC CONDITIONS

Pasteur (1885) was the first to ask the question as to whether animal life is possible without the aid of microorganisms. He believed that the question would be answered in the negative.

Nencki (1886) brought up theoretical considerations supporting the opposite view, reasoning that various digestive enzymes of the pancreas, stomach, and intestine split up the food into nourishing end products without the aid of bacteria, and that many or most of the end products of bacterial decomposition in the intestine, such as indol, skatol, phenol, fatty acids, aromatic acids, ammonia, organic bases (ptomaines), carbon dioxide, hydrogen, hydrogen sulfide, and carbon monoxide, are not foods, and are actually harmful to the organism. He predicted that chickens, dogs, rabbits, or guinea pigs might be successfully grown aseptically, but he did not make any experimental tests.

Eleven years later (1897) Nuttall and Tierfelder succeeded in raising sterile guinea pigs for about a month. These were obtained by Caesarian operation. Control guinea pigs were also obtained by Caesarian operation, but were not kept aseptic. By noting the change in weight these authors concluded that animal life is possible under completely sterile conditions. Because the experiment was terminated in such a short time, owing to technical difficulties, the results have been criticized.

Schottelius (1899-1913) in a series of experiments reached opposite conclusions with respect to chickens brought up under aseptic conditions. Schottelius believed that the decline and death of the animals fed on sterilized food was due to disturbances of digestion and loss of weight. It was only necessary to add *Bacillus coli* to their food to enable the chickens to regain their vigor and develop normally. Kianizin has criticized this interpretation on the ground that animals must lose a greater weight to die of hunger alone.

In 1901 Mme. O. Metchnikoff raised sterile tadpoles of the frog, *Rana temporaria*. The outside of the gelatinous mass was washed repeatedly in sterile water. The embryos were removed with sterile pipettes, passed through a series of vessels of sterile water, finally to

culture bottles where they were left to the end of the experiment. When bread was the sole food used in the experiment, whether it was sterile or not, the development of the tadpoles was much slower than under normal conditions. At the end of eighty days, only small rudiments of hind legs were present in the best of the controls (but in none of the experimental animals), whereas normally the tadpoles are completely transformed by this time. It is interesting to note that numbers of the tadpoles which were not aseptic died during the first few days. Others showed contamination after a longer period and these served as controls. All had died by the seventy-ninth day, but the aseptic tadpoles died more slowly. The controls, however, showed the more rapid development and growth, and therefore Mme. Metchnikoff concluded that microbes are necessary to the development of tadpoles.

Moro (1905) repeated the experiments of Mme. Metchnikoff, using tadpoles of the toad, *Pelobates fuscus*, with the same results. He used as food an emulsion of pulverized wafers and finely divided white of egg. Experimental animals were less than half as large as controls and did not develop pigment.

This same year, 1905, Portier pointed out that certain insects in a state of nature because of their manner of living are not contaminated during their period of development. The larvae of certain Microlepidoptera hatch out of eggs entirely surrounded by a gelatinous mass, and burrow directly through the epidermis into the leaf. By carefully washing the surface of the leaf with hydrogen peroxide and extracting the larvae aseptically, Portier found that one-third of the larvae of *Lithocolletis* (of the oak, the elm, and *Prunus Padus*) were aseptic, the others being contaminated in part with bacteria, in part with fungi, particularly *Aspergillus niger*. The larvae of *Nepticula* (of the rose) were always aseptic.

It is also of interest to note that later workers have found other forms of animal life in a state of nature devoid of, or at least containing very few, bacteria during certain periods. Weinberg and Mlle. Inga Soeves (1906) have obtained bacteria only exceptionally from the contents of the whip worm, pin worm, and hookworm of man and the chimpanzee.

Metchnikoff (1903, 1908) developed extensive theories as to the harmfulness of bacteria in the digestive tract of man and other animals, believing that life would be prolonged in the absence of such infection.

Bogdanow (1906, 1908), working with the larvae of *Calliphora vomitoria*, arrived at the conclusion that the aid of microbes is necessary to their development. In the best developed case, the sterile larvae attained a length of .8 cm. and died without pupating, while, up to 1906, he had only one case of sterile larvae, the others being contaminated with cocci, which, according to the author, were contained in the eggs themselves. These larvae the author considered as sterile in comparison with the larvae which developed in the presence of proteolytic bacteria.

In later experiments Bogdanow had better results. He frequently obtained sterile larvae, and once the larvae attained normal length, and one sterile fly hatched out, apparently perfectly normal. Bogdanow had the ingenious idea of adding trypsin to some of the sterile cultures. Under these conditions the larvae developed, in general, very well, and Bogdanow therefore concluded that, in sterilized meat, the larvae of *Calliphora* develop very poorly, but if proteolytic bacteria are added, or trypsin, development is more nearly normal.

Wollman (1911) repeated this experiment, and also studied the effects of certain pure cultures of microbes on the development of the larvae of *Calliphora vomitoria*. The experiment included more than three hundred eggs. Frequently aseptic larvae reached a normal length and weight. Sterile larvae move slowly and crawl indifferently over the meat, or the walls of the tube. The difference in length between them and the controls is accentuated the first few days, then the difference tends to disappear, and the aseptic larvae attain normal proportions only a little later than the controls. Individual differences between sterile larvae are partly accounted for by the differences in the production of ferments. The difference between the sterile larvae and the controls is believed to be induced by odor of the decomposing meat, which doubtless excites the larvae to eat. When meat is sterilized by Tyndalization, the aseptic larvae differ but little from the controls.

When the larvae were grown with certain pure cultures of bacteria, the putrefying bacteria only were harmful, the others giving as good results as the controls. The conclusion of Bogdanow is that *Calliphora vomitoria*, which in its natural state lives entirely in association with bacteria, may live without the intervention of microorganisms.

Kianizin (1916) reviewing earlier work, concluded that, in mammals, sterilizing the air and food supplied them causes decreased nitrogen assimilation and death. He came to the conclusion that the

microorganisms contained in the air are absolutely necessary for normal metabolism and for life. The decrease of nitrogen assimilation is accompanied by an increase of leucomaines. He showed that birds (pigeons) live longer than mammals in sterile conditions. He compares his work with that of Charrin and Guillemonat in 1901, who observed the effect on guinea pigs of sterilizing the air and the food. Their apparatus was arranged so that the experimental and the control animals, two to four at a time, could be observed simultaneously. The results obtained by adding the figures of the different series showed that 70 per cent (19 out of 27) of the experimental animals died, while 34 per cent (10 out of 29) of the controls died. The average of the daily loss of weight was greater in the experimental animals. Also the ratio of nitrogen in the urea to total nitrogen in the urine was less than in the controls.

In 1912 Cohendy experimented, as Schottelius had done, on chickens obtained from sterilized eggs. He compared the general condition and weight of (a) sterile chickens supplied with sterile air and food, (b) chickens from sterilized eggs, infected, and then fed with sterilized food and kept in conditions similar to those of (a), and (c) chickens treated normally. The chickens of both (a) and (b) grew much less and were weaker than the normal chickens, but he states that there was no great difference between the chickens of (a) and (b), and he concluded that a complete absence of microorganisms does not in itself entail any deterioration in higher vertebrates. The sterile chicks had good resistance to death from cold, humidity, thirst, and hunger, thus showing the active rôle of infection when resistance is lessened by a physical cause.

In reviewing these experiments in the light of modern biochemical knowledge, it may be noted that most of the unfavorable results of attempts to grow animals aseptically may be interpreted as the result of inadequate food. For instance, sterilized bread, used by Mme. Metchnikoff for tadpoles, and even the combination of bread and egg-white used by Moro, is notably deficient in certain amino acids and in vitamines. Beginning with Guyénot's studies in 1911, the problem has been approached from this aspect, with more favorable and conclusive results. The studies of Northrop in 1916 on the nutrition of *Drosophila* throw some light on the possible insufficiencies of the food conditions in Bagdanow's experiment on *Calliphora*, since certain accessory food substances must be present in order for fly larvae to utilize other food.

ASEPTIC DROSOPHILA

Aseptic *Drosophila melanogaster* (*ampelophila* Löw) were first grown by Delcourt and Guyénot (1911). The start of the aseptic line was secured by dilution, that is, by transferring a female rapidly from one aseptic bottle to another, allowing time for one or two eggs to be deposited in each bottle. The line thus established was maintained for more than forty generations, involving at least 400,000 flies. The organisms showed no diminution of vigor or of fecundity; on the contrary, their development and reproduction was more effective than in the optimal conditions found in nature. Mortality of larvae and pupae in aseptic cultures was practically null.

Guyénot used this aseptic line for a series of detailed studies, published in 1913, showing that:

(a) Dead yeast in aseptic media may take the place of living yeast and other microorganisms in the state of nature, as the principal food for the flies.

(b) Sterilized potato without yeast is an inadequate food, but flies showed individual differences in ability to adapt themselves to this medium, and, in proportion to the number of generations, more and more favorable results were obtained. It therefore seemed that the organisms became more adjusted to it.

(c) Flies grown as larvae on sterilized yeast are sexually mature when they hatch, and females begin egg-laying at once. If, however, the larvae are grown on sterile potato, sexual maturity of both males and females is delayed for seven to fifteen days. If such flies are transferred to yeast on hatching, sexual maturity is reached in a shorter time.

(d) Fecundity of flies depends not only on how they are nourished as larvae, but also on the food they receive as adults.

(e) Females grown on yeast, fertilized, then transferred to potato, lay a few fertile eggs, then eggs which do not hatch. The spermatozoa in the seminal receptacle of the poorly nourished females undergo reabsorption. Such a phenomenon permits the nullification of the effects of the first mating and makes possible a second mating with a different male.

(f) Egg-laying is accomplished as a discharge caused by the superabundance of formed eggs. It occurs more or less rapidly according to whether the female is well or poorly nourished. Great individual differences occur in the time that laying begins in the

absence of the definite stimulus (fertilization). A sudden change in medium or in temperature causes an immediate cessation of laying; the females die with enormous abdomens. Sometimes, in eggs already fertilized, thus retained, development continues and thus an accidental viviparity is produced.

Aseptic *Drosophila* were grown in 1916 by Loeb and Northrop. They sterilized the eggs in one per cent $HgCl_2$ or a saturated solution of $HgCl_2$ in alcohol, for six or seven minutes. They studied growth and metamorphosis on different media and concluded that yeast was the only sufficient food for them, and that these flies are probably monophagous insects.

In 1917 these same authors studied the influence of food and temperature on the duration of life, concluding that:

(1) With a supply of proper and adequate food the duration of the larval stage is an unequivocal function of the temperature at which the larvae are raised and the temperature coefficient is of the magnitude of that of a chemical reaction, i.e., about two or more for a difference of $10^{\circ} C$. It increases at the lower and is less at the higher temperatures.

(2) The duration of the pupal stage of the fly is also an unequivocal function of the temperature. The coefficient is identical with that for the larval period.

(3) The same statement is true for the imago.

Temp.	Larval stage	Pupal stage	Imago	Total
$10^{\circ} C$	57 days	die	120.5 days	$177.5+x$
15	17.8	13.7	92.4	123.9
20	7.77	6.33	40.2	54.3
25	5.82	4.23	28.5	38.5
27.5	4.15	3.20
30	4.12	3.43	13.6	21.15

The authors suggest that the duration of life is determined by the production of a substance or substances leading to old age and natural death, or by the destruction of a substance or substances which normally prevent old age and natural death.

In making the studies on the duration of imaginal life, the aseptic flies were transferred to glucose agar as they hatched, since, according to the experimental results of these authors, the imago is able to live the normal length of time on food which contains no yeast and which is inadequate for the growth of the larvae.

Northrop (1917) using the thirtieth aseptic generation of these flies studied the effect of prolongation of the period of growth on the

total duration of life. By adding yeast extract at different periods after the hatching of the larvae, the egg-larval-pupal period could be increased from 8 to 17 days. The imaginal period was not affected by this difference, but was practically the same in every case, 10.5 to 11.7 days, at 27.5° C on glucose agar.

Northrop also found that the number of flies which are able to develop on a definite quantity of yeast may be increased by the addition of banana, casein, or sugar to the yeast. Yeast therefore contains a sufficient excess of the necessary (accessory) substances to render available as food approximately twice its weight of banana. Kidney, liver, and pancreas from the dog, and liver from the mouse, as well as the bodies of flies themselves are an adequate source of food for the larvae, whereas on other tissues used no growth takes place. Tethelin was found ineffective in stimulating growth.

In 1926 Northrop studied carbon dioxide production in relation to the duration of life of *Drosophila* cultures. In this he used the 195th-206th generations of this same aseptic strain, started in 1916. He found more carbon dioxide to be produced at 15° C than at 26° C in the dark. Illumination, however, markedly increased the CO₂ output. There was no evidence of a diminution in CO₂ output toward the close of the life-span of the imagoes, thus contradicting the "energy limit" idea of Rubner.

Incidentally, Northrop mentioned that a progressive decline in duration of life of this aseptic strain had been noticed. No explanation is suggested by the author, but it seems reasonable that this might be due to the fact that the original start of the aseptic line was heterozygous, and that the successive generations were maintained by mass transfers (not single pair brother and sister matings). A gradual but slow progressive decline in the longevity might result as the flies approached homozygosity. This interpretation would be consistent with the results of Castle, *et al.* (1906), and of Moenkhaus (1911), on the effects of inbreeding *Drosophila*, with reference to fertility, and also with the statement of Delcourt and Guyénot that their aseptic flies after forty generations showed no diminution of vigor or of fecundity.

Baumberger (1919) showed that there is a correlation between concentration of yeast and length of the larval period of aseptic flies, but believed nucleo-protein to be the essential thing, since occasionally insects matured on banana, when this food was sufficiently concentrated.

Bacot and Harden (1922) grew aseptic larvae of *Drosophila* to determine what, if any, vitamines are essential to their growth. Vitamin B added to a basal diet of caseinogen, starch, and salts, and cane sugar was found to be very essential, and could be supplied from wheat germs, when given with butter fat (source of Vitamin A?), although not so well as from yeast. Evidence for the necessity of Vitamin A was insufficient. It would be necessary to see if the larvae would grow in the presence of a fat minus this vitamin. Vitamin C, added in the form of lemon juice, was not required.

DURATION-OF-LIFE STUDIES ON DROSOPHILA

(Not under aseptic conditions.)

A number of studies on the duration of life of *Drosophila* have been made, which do not involve asepticity. Moenkhaus (1911) makes the earliest statement to the effect that "we have kept females alive 153 days," but he gives no details.

Hyde (1913) crossed truncated *Drosophila* with the long-winged or wild type, and found that the F_1 had a higher duration of life than either parent type, and were more vigorous.

The hybrids average 47 days, while the truncated parents had a mean duration of life of 21.4 days, and the inbred wild type of 37.4 days. In both parent stocks the males lived longer than the females; for the truncate the difference was 8 days, and for the wild, 6 days. The same condition with respect to differential sex mortality held for the F_1 and the F_2 .

Lutz (1911) found a slight negative correlation between length of adult life and duration of embryonic periods. He also studied the life of starved flies given water but no food.

In the Biology of Death, Pearl (1922) makes a comparison of 70 flies grown aseptically by Loeb and Northrop at 25°C with 5,936 of his flies of all genetic lines, as to duration of life. This gives a difference of 3.7 days in favor of the non-sterile flies. However, since the flies were not of the same inbred stock, the comparison is open to criticism.

A series of studies by Pearl and Parker (1921-24) and by Gonzalez (1923) led to the following conclusions:

(1) The life curve for *Drosophila* males resembles that of human males of today, and still more closely that of males of the Roman provinces in Africa at the beginning of the Christian era.

(2) There are hereditary differences within the same species of *Drosophila* with respect to duration of life, which when isolated show a high degree of permanence.

(3) Successive etherizations have no sensible effect on the duration of life of *Drosophila*.

(4) There is, for a given capacity duration-of-life bottle, an optimal density below and above which the duration of life is decreased.

(5) In the wild and vestigial strains, the females have a greater expectation of life than the males.

(6) Under conditions of complete starvation, the females still have a greater expectation of life than the males, namely four to five hours.

Gonzalez has isolated the five mutant strains that compose quintuple stock, and studied the effect of each gene separately, and in various combinations with the other four, on the duration of life, sex mortality, and fertility. He showed that the character vestigial is responsible for most of the shortened duration of life of quintuple stock. Black, and to a greater degree, speck, have a longer duration of life than the wild type, suggesting that here the presence of a mutant factor increases longevity.

In black there is no significant difference in mean duration of life of males and females, while in purple and in speck the males live longer, and in vestigial, and in arc, the females live longer. Average progeny per fertile mating shows that purple is a strain having unusually high fertility, while in vestigial the fertility is low.

Using two or more of these mutants in combination Gonzalez concluded that the number of mutant genes in a strain has no relation *per se* to its duration of life, but rather the intrinsic properties of the mutations present exert an effect. The result is not an algebraic sum of two effects, but may be greater or less than either extreme, or intermediate.

MATERIALS AND METHODS

Six strains of *Drosophila melanogaster* were used in the experiment, including the wild type and five recessive mutants:

White eye, sex-linked.

Yellow body color, sex-linked.

Cinnabar eye, II chromosome.

Vestigial wing, II chromosome.

Curled wing, III chromosome.

These flies were secured from the Department of Genetics in August, 1925, and inbred, single pair brother and sister matings, for a year, thus passing through twenty-six generations before the experiment was started. The stocks were believed to be as homozygous as it is possible to make them, thus rendering heredity a constant, so that differences in length of life can be fairly attributed to the different environmental conditions to which the flies were subjected.

The stock cultures were maintained on the ordinary culture medium:

100 grams mashed banana

100 cc. H₂O

4 grams agar

The agar was dissolved in the water, the bananas added, and the whole allowed to come to boil. The medium was poured into milk bottles of one-fourth pint capacity, to a depth of three-fourths of an inch, and a minute quantity of dry magic yeast sprinkled on the surface. All bottles and plugs used in the entire experiment were sterilized in hot air at 150° C for one hour before they were used.

Flies which were to be used as controls were hatched from this same culture medium and fifty were transferred to each duration-of-life bottle. In these milk bottles of one-fourth pint capacity, Pearl's synthetic medium was used. This medium, described by Pearl (1926, p. 513), has the following composition:

Solution A	Grams
Cane sugar	500
KNaC ₄ H ₄ O ₄ ·4H ₂ O	50
(NH ₄) ₂ SO ₄	12
MgSO ₄ ·7H ₂ O	3
CaCl ₂	1.5
H ₂ O to make 3000 cc. of solution.	

Solution B	Grams
Agar-agar .	135
Tartaric acid ($C_4H_6O_6$)	30
KH ₂ PO ₄ .	6
H ₂ O to make 3000 cc. of solution.	

In my experiment the agar was not added until the medium was needed for the duration-of-life bottles. Solutions A and B were made up separately and sterilized in the autoclave, then mixed in a proportion of one to one. According to Pearl this medium has a pH of 3.7, and on this account there is practically no contamination of the cultures by bacteria. This I also found to be true, but, owing to the readiness with which molds grow upon it, this medium was not found satisfactory for stock cultures or breeding bottles under the conditions existing in the laboratory. It was highly satisfactory for the duration-of-life bottles, however, since the flies were transferred to fresh media every other day.

The start of aseptic flies was secured from eggs laid by the first sets of controls. After the flies had been on the media forty-eight hours, and had been transferred to new bottles, the cakes of media in the bottom were removed and placed under a dissecting binocular. Here the eggs were picked up, some twenty or thirty of them, on the point of a scalpel blade, and transferred to very small watch crystals containing a saturated solution of mercuric chloride in 70 per cent alcohol, where they were allowed to remain about seven minutes. The eggs were then transferred to shell vials containing aseptic media. This is a slight modification of the method described by Loeb and Northrop (1916). All apparatus used in handling the eggs was first sterilized in hot air at 150° C for one hour.

The medium used in the aseptic bottles had as a foundation the banana agar of the stock culture bottles, to which was added 10 per cent of Fleischmann's yeast.

250 grams mashed banana
 250 cc. H₂O
 10 grams agar
 50 grams (4 cakes) Fleischmann's yeast

The banana was added after the agar was dissolved; the yeast, mixed with a little water, was added last, and the whole mixture boiled for five minutes. The medium was then poured into vaseline bottles of one-fourth pint capacity, to a depth of three-fourths of an inch. These were used in preference to milk bottles because of the wide neck, against which the cotton plug could be fitted securely. Plugs were

made similar to those described by Delcourt and Guyénot (1911), but instead of the elaborate glass stopper described by them, pipettes, sealed at the lower end, were used, and around these the cotton was wrapped securely. After the plugs were inserted a paper cap was tied over the top, to keep the cotton from being wetted by the condensing steam as the autoclave cooled, and also to prevent dust from settling on the surface of the cotton, where it might the more readily cause contamination when the flies were introduced or removed. A doctor's and nurse's model portable autoclave was used in the latter part of the experiment, and the medium sterilized one hour at seven pounds pressure on each of four successive days.

When the eggs sterilized in mercuric chloride were first introduced it was, of course, necessary to remove the entire plug, and for this reason shell vials were used because of their smaller aperture. After that, the vaseline bottles, having the same capacity as the control milk bottles, were used exclusively.

The aseptic flies that hatched out were transferred to new aseptic bottles by means of a long glass pipette. A double thickness of voile was tied securely over the large end, each tube wrapped in a separate towel, and sterilized in hot air. These pipettes were of the same diameter as the smaller sealed stoppers extending through the cotton plugs. Rubber tubing of a diameter to fit securely over the pipettes, was cut in 16 or 18-inch lengths and kept in 95 per cent alcohol. The alcohol keeps the rubber aseptic and pliable, and has the advantage of drying very quickly when the tubing is needed for making a transfer. The dry rubber tube was fitted over the end of the sterile pipette. The bottle containing the aseptic flies was held in a horizontal position while the glass stopper through the cotton plug was removed, and the pipette inserted. The flies were drawn up into the pipette, counted as this was done, and transferred quickly to a fresh bottle. The glass stopper was passed through an alcohol flame before it was replaced. A fresh tube was used for each group of flies transferred.

All experiments were carried on under constant temperature, 25° C., and for this purpose an electric incubator was used. This was kept under careful observation, and the amount of fluctuation observed to be not more than $\pm 0.5^{\circ}$ C. The humidity was kept constant by placing open pans of water in the incubator.

Since the medium used for *Drosophila* food is in itself a nutrient agar, as a general rule any contamination in the aseptic bottles showed itself plainly on the surface, and such bottles were discarded at once.

However, the bottles containing the aseptic flies which had died since the last transfer were sent to the Department of Bacteriology and given a rigorous test. Nutrient broth containing glucose and beef extract was poured into the bottles, which were then incubated for twenty-four hours at 37° C. Transplants were then made to beef extract agar, and incubated for six or seven days at the same temperature. Of 224 bottles tested, only 8 had to be discarded because they showed contamination, the rest remaining aseptic.

RESULTS

The first set of controls, started in August, 1926, from stock which had been inbred 26 generations, involved 8,461 flies, as shown in table 1 (summarized from tables 21-27), distributed among the six strains. The lowest average duration of life was shown by the vestigial flies, 16.99 days, and the highest by yellow, 26.28 days. The average for all six strains was 22.13 days.

Experiment I, which was started just a little later, involved 3,664 flies which were grown aseptically until the time the adult flies emerged, then run in duration-of-life bottles in the same manner as the controls. These flies were two and three generations removed from the first set of controls, since eggs from the six control lines were sterilized to get the start of the six aseptic lines. However, since it requires between two and three months to run through any given set of flies, the life-span of the two sets overlapped for the greater part of the time, and the results are therefore comparable. The mean duration of life for this set was 29.81 days, as shown in table 5 (summarized from tables 42-48), and the effect of the aseptic larval and pupal life seems, therefore, to increase the expectation of life at emergence, in this case by 7.68 days, a difference so much greater than the probable error as to be obvious at sight. The increase was remarkably uniform in all six types, ranging from 5.77 days in the wild, to 9.45 days in the white, the others falling into intermediate positions.

	Cinnabar	Curled	Vestigial	White	Wild	Yellow	All six types
Experiment I	32.95	26.86	23.70	33.59	28.32	33.64	29.81
Controls	25.54	20.98	16.88	24.14	22.55	26.28	22.13
Difference	7.42	5.88	6.82	9.45	5.77	7.36	7.68

Tables 15 and 18 give the number of flies surviving every third day on the basis of 1,000 starting imaginal life at the same time. These

data are plotted on arith-log paper in figure 1 and show that the flies which had been kept under aseptic conditions during the larval-pupal period have a greater number of survivors than the controls at every point of the life curve.

In Experiment II the aseptic flies were transferred as they hatched to aseptic duration-of-life bottles containing banana agar but no yeast. This food proved to be inadequate, in comparison with that used in Experiment III. The mutant strains showed individual differences in regard to their ability to survive on this medium, as shown in table 6 (summarized from tables 49 and 50). Cinnabar had an unusually high death rate from accidental deaths, as many flies were observed to be caught between the medium and the sides of the bottle. Yellow did not do so well as in Experiment I, curled averaged practically the same, while white, vestigial, and wild averaged considerably higher. The mean duration of life for the whole group of 2,912 flies was 31.31 days. Separate records for the mortality of the two sexes were not kept, because of the difficulties involved. On the basis of 1,000 flies starting imaginal life the same day, table 19 gives the number of flies of each type surviving every third day. Life curves of flies in Experiment II are plotted in figures 2-8.

In Experiment III the aseptic flies were kept on banana agar with 10 per cent yeast throughout imaginal as well as larval and pupal life. Hatches over a twenty-four-hour period were transferred in groups of fifty to aseptic duration-of-life bottles. In some of the bottles males and females were kept together, while in the greater number the males and females were isolated. This latter method facilitated the counting and recording of deaths and reduced the number of transfers necessary. A remarkable increase in the mean duration of life was found in each of the six lines, most strikingly in the vestigial flies, in which, compared with control conditions, the duration of life was increased more than 200 per cent in Experiment III (40.55 days). When yeast is used in a concentration of 10 per cent, the aseptic food medium seems to be adequate in every respect. It is possible that some of Vitamins A and C are present in the banana, while Vitamin B which Bacot and Harden have shown to be so essential is abundantly present in sterilized yeast. The experiment involved 4,668 flies, which had a mean duration of life of 44.2 days, as shown in table 7 (summarized from tables 51-57). Differential viability in the six strains tended in large measure to be effaced under aseptic conditions (table 20, and figs. 2-8).

A second set of controls was started in December, aseptic flies being transferred directly to ordinary breeding bottles. The offspring from these flies were treated as the first set of controls had been, and were run while Experiment III was in progress. A considerable increase in mean duration of life was found when these flies were compared with the first set of controls, the average for the second set of 5,371 flies being 32.51 days, table 2 (derived from tables 28-34), an increase of 10.38 days. This increase might be due to any of the following causes:

(1) Seasonal. The first set of controls were run in the late summer and early fall, when the air was full of dust carrying molds and bacteria. Considerable difficulty was found in keeping the breeding bottles reasonably free from these growths and in getting good hatches. In December and January, this situation was much improved.

(2) Difference in ancestry. The first set of controls came from flies carrying a heavy load of bacteria, yeasts, and molds; the second, while continuing the same direct line, came from flies that had lived under aseptic conditions for six or eight generations.

(3) Possible mutations affecting duration of life, in a positive manner.

For purposes of comparison with experimental flies, and for plotting survival curves, the two sets of controls have been averaged (tables 3 and 4, derived from tables 35-41). Since the experiments of necessity extended over several months and involved a number of generations, it is thought that the unfavorable conditions for the first set of controls may be balanced by the favorable conditions of the second set, the two together thus giving a more accurate mean for the duration of life under control conditions, than would either alone. In comparison with the 13,832 control flies which averaged 26.16 days, the aseptic flies of Experiment III averaging 44.2 days show an increase of 18.04 days (table 7).

With respect to the differential death rate of the two sexes it may be stated that:

Yellow (table 13), which is a sex-linked recessive character, shows a greater expectation of life for the females in every experiment, the difference being from 3.68 to 7.5 days. However, white (table 11), which is also a sex-linked recessive, shows very nearly equality in the expectation of life of males and females in the control series. The same condition holds for Experiment I, but in Experiment III the males live longer than the females by 5.49 days.

Again, among the second chromosome mutants, cinnabar (table 8) and vestigial (table 10), we have disagreement. The cinnabar males have the greater expectation of life in every experiment, from 5.1 days in Experiment III to 10.42 days in Experiment I, and over 9 days in the controls. Vestigial, however, shows a greater expectation of life for the females in the controls of 4.13 and 10.02 days respectively, and in Experiment I by 9.62 days. In Experiment III the ratio is reversed and the males outlive the females by 3.65 days.

Curled (table 9), a second chromosome character, shows a greater expectation of life for the males of 3.7 and 4.76 days in the control experiments, equality in Experiment I, and a reversal of conditions in Experiment III, in which the females lived longer by 4.77 days. This difference in Experiment III can be at least partly explained by the observed greater number of accidental deaths among the isolated males.

Wild (table 12), shows a slightly greater mean duration of life for the males under control conditions (1.4 days), but the females live longer by 5.55 days in Experiment I, and by 1.76 days in Experiment III.

DISCUSSION

The differential sex mortality ratios among the flies in Experiment III, while significant from the standpoint of numbers and probable errors, may not always indicate differences in vitality.

(a) The sexes were, for the most part, isolated, and no two bottles represented exactly the same conditions. There were minute differences in moisture content, and the rate of shrinkage of the media; the occasional presence of drops of water on the surface of the medium; accidental deaths due to the fact that the flies were caught between the food and the sides of the bottle. The last cause in itself, as already mentioned, is sufficient to explain the apparent reversal of the sex mortality of the curled flies, since the isolated males were observed to meet accidental death in this manner in greater numbers than was true for the females, or for any other type of flies.

(b) Isolation may affect the females unfavorably, by removing the stimulus to egg-laying. The greater number of females had not copulated during the twenty-four-hour period before they were transferred to duration-of-life bottles. Guyénot has stated that in the

absence of a definite stimulus egg-laying may cease, and the females die with enormously swollen abdomens. This was occasionally observed in my experiment.

Under control conditions, however, the sex mortality ratios may truly represent differences in vitality, especially of resistance to infection, as is evidenced by the high death rate of the females in cinnabar and curled, and of the males in vestigial and yellow.

By comparison of the sex ratios in the wild type with those of the sex-linked recessives white and yellow, it does not seem probable that recessive characters *per se* in the X chromosome, being simplex in the male, cause a lowering of the expectation of life among the males. This latter view is expounded by Geiser to account for the numerous instances of such low sex ratios among animals in which the male is the heterozygous type. The intrinsic qualities of the mutant genes, in relation to the whole chromosome complex, seem to determine whether the males or the females have the greater expectation of life, and this expectation may be modified or even reversed under different environmental conditions. In this respect my results are in accord with those of Gonzalez (1923), who studied separately each of the mutations composing the quintuple stock (five second chromosome mutations) used by Pearl and Parker in previous experiments.

A much higher effective fertility on the part of the aseptic flies as compared with controls was noted, confirming the results of Delcourt and Guyénot. Mortality among larvae and pupae was reduced to a minimum. Even among the vestigial, curled, and white flies which often do not give good yields under control conditions, the hatches (from twenty-five to fifty parents) averaged about one hundred and fifty per twenty-four-hour period.

The larval-pupal period was reduced to a minimum for this temperature, making hereditary line differences apparent. Cinnabar, yellow, and wild flies had to be transferred on the eighth day, since the next generation began emerging on the ninth day. White-eyed flies hatched on the tenth day, curled and vestigial on the eleventh. Here we find roughly a negative correlation between the rate of larval development and the length of life, confirming Weismann's criticism of the Buffon-Flourens theory that duration of life is a multiple ($\times 6$) of the developmental period. Here the flies that emerge last, vestigial and curled, have the shorter duration of life, which seems to suggest a correlation of short life and slow development with low vitality, rather than rate of energy production.

Accidental deaths have not been eliminated in Experiment III—in fact these were observed in considerable numbers. Nevertheless, the life curves (figures 2–8 on arith-log paper) show a much nearer approach to the right angle than do the controls. Theoretically, of course, if all the flies were homogeneous with respect to hereditary factors, and were subjected to exactly the same environmental conditions, deaths would be expected to occur for all flies at very nearly the same time. This was occasionally noted for individual aseptic bottles, but, as the conditions for any two bottles were not exactly the same, since the flies were so fragile and there were many fortuitous causes of death, a greater variation in the time of deaths resulted.

Under control conditions, the differences in mean duration of life among the six types range up to 15 and 20 days, but under aseptic conditions the greatest difference is less than 9 days. This, as well as the actual increase in mean duration of life of all strains in Experiment III, is largely accounted for by the striking reduction of deaths in the short-lived strains during the early days of imaginal life. The spread of the control and aseptic survival lines, plotted on the basis of 1,000 starting life on the first day, brings out these differences more clearly than words. The data from which these figures are drawn are found in tables 17–20, which are derived from tables 35–57.

Both weighted and unweighted means are given in summary tables (1–14), so that the effect of differences in numbers among the six varieties in the successive experiments may be accurately judged.

Calculations of the standard deviations were made, so that the coefficients of variation for the different experiments could be compared.

	Sigma	Mean	Coefficient of variation
Controls	12.80	26.16	$48.92 \pm .20$
Experiment I	12.61	29.81	$42.30 \pm .33$
Experiment II	11.23	31.31	$35.86 \pm .32$
Experiment III	15.35	44.20	$34.72 \pm .24$

Thus we find a progressive decline in the coefficients of variation from the controls to Experiment III, which is consistent with the greater approach of the aseptic flies to the right angle type of life-curve.

CONCLUSIONS

Six strains of inbred *Drosophila melanogaster* totaling over 25,000 flies were studied under aseptic and control conditions. The results show that:

1. Aseptic larval-pupal life, followed by control conditions during the imaginal stage, increased the expectation of life of adult flies by 34.7 per cent of the life-span in comparison with the first set of controls, and by 13.7 per cent in comparison with the average of the two sets of controls.
2. Sterilized banana agar is an inadequate food for aseptic *Drosophila* imagos, although the mean duration of life is higher than for the controls.
3. On adequate food, banana agar and ten per cent yeast, *Drosophilas* reared throughout life under aseptic conditions have a greater mean duration of life than the controls by 18.04 days, 69 per cent of the life-span.
4. The life-curves of the aseptic flies (plotted on the basis of 1,000) show a greater approach to the right angle type, than do the controls.
5. Differential viability in the six strains of flies under aseptic conditions tended in large measure to be effaced.
6. No uniformity was found with respect to differential sex mortality, though yellow shows a consistently greater expectation of life for the females, and cinnabar for the males.
7. The coefficients of variation show a progressive decline from the controls through Experiments I and II, to Experiment III.

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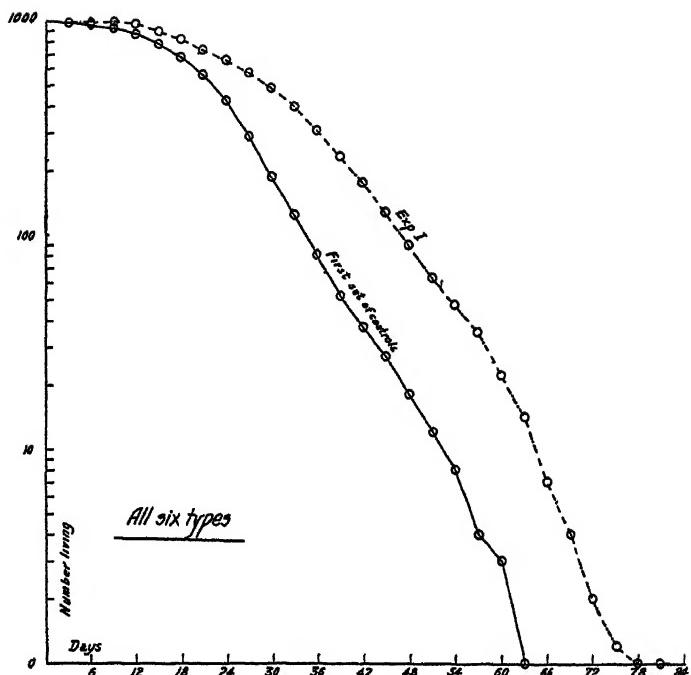


Fig. 1. All six types. Life-curves of imagoes of first set of controls, and Experiment I. In Experiment I, flies were aseptic as larvae and pupae only, and the imagoes were run in the same manner as the controls.

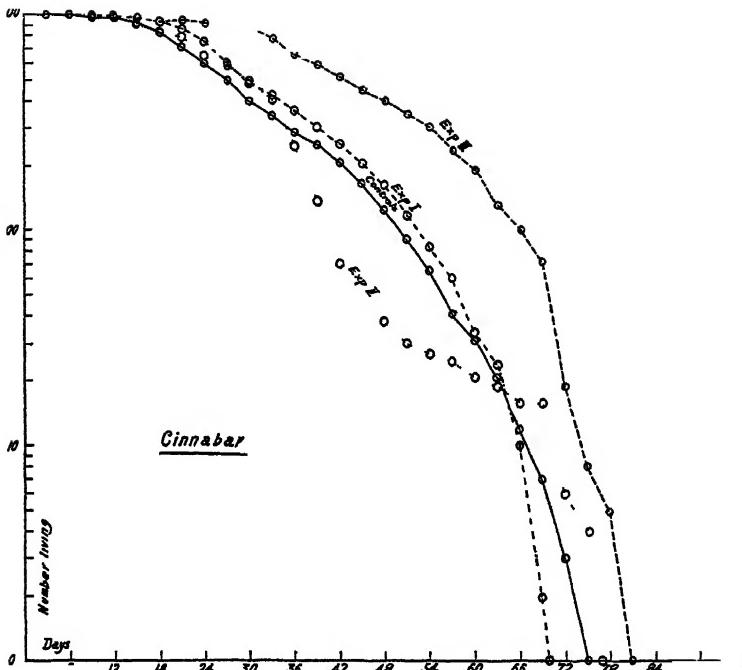


Fig. 2. Cinnabar. Life-curves of imagos.

Controls, first and second sets together.

Experiment I, flies aseptic as larvae and pupae, imagos run in same manner as controls.

Experiment II, flies aseptic throughout life; larvae on banana agar with 10 per cent yeast, imagos on banana agar without yeast.

Experiment III, flies aseptic throughout larval and imaginal life, on banana agar with 10 per cent yeast.

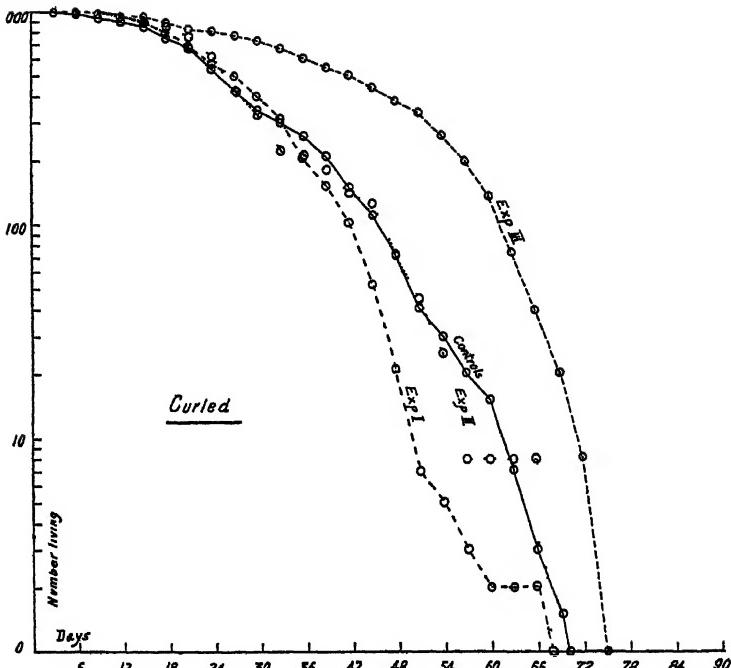


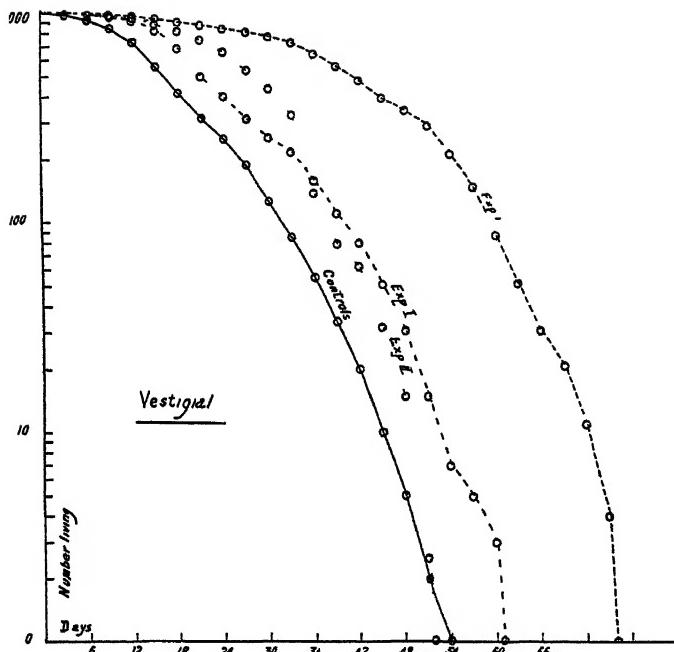
Fig. 3. Curled. Life-curves of imagos.

Controls, first and second sets together.

Experiment I, flies aseptic as larvae and pupae, imagos run in same manner as controls.

Experiment II, flies aseptic throughout life; larvae on banana agar with 10 per cent yeast, imagos on banana agar without yeast.

Experiment III, flies aseptic throughout larval and imaginal life, on banana agar with 10 per cent yeast.

Fig. 4. *Vestigial*. Life curves of imagoes.

Controls, first and second sets together.

Experiment I, flies aseptic as larvae and pupae, imagoes run in same manner as controls.

Experiment II, flies aseptic throughout life; larvae on banana agar with 10 per cent yeast, imagoes on banana agar without yeast.

Experiment III, flies aseptic throughout larval and imaginal life, on banana agar with 10 per cent yeast.

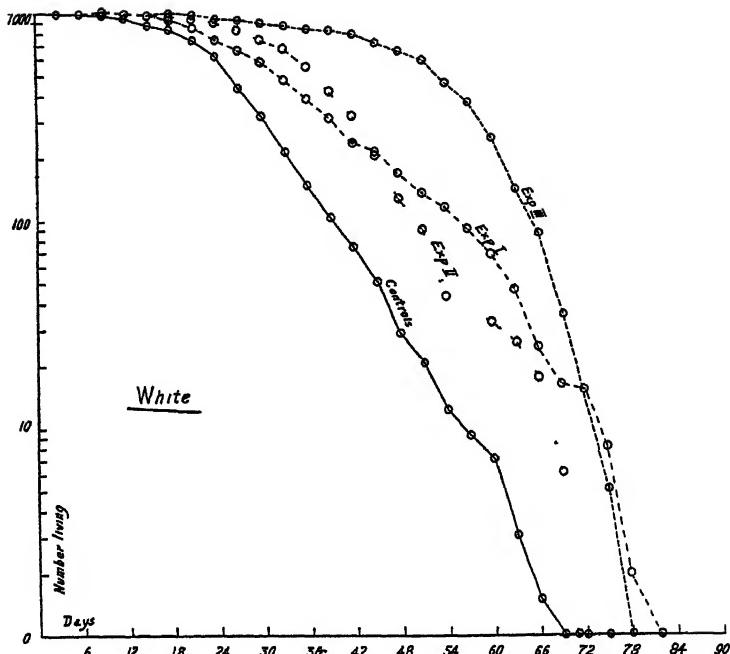


Fig. 5. White. Life curves of imagos.

Controls, first and second sets together.

Experiment I, flies aseptic as larvae and pupae, imagos run in same manner as controls.

Experiment II, flies aseptic throughout life; larvae on banana agar with 10 per cent yeast, imagos on banana agar without yeast.

Experiment III, flies aseptic throughout larval and imaginal life, on banana agar with 10 per cent yeast.

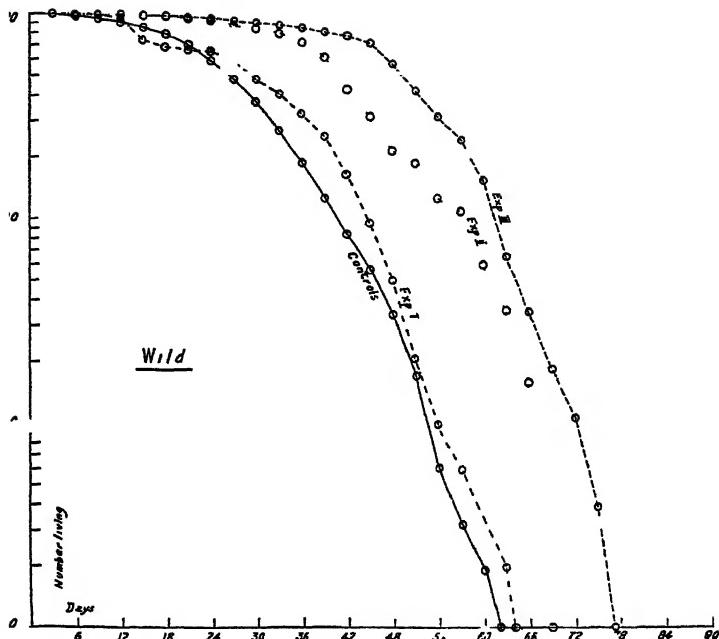


Fig. 6. Wild. Life curves of imagoes.

Controls, first and second sets together.

Experiment I, flies aseptic as larvae and pupae. imagoes run in same manner as controls.

Experiment III, flies aseptic throughout life; larvae on banana agar with 10 per cent yeast, imagoes on banana agar without yeast.

Experiment III, flies aseptic throughout larval and imaginal life, on banana agar with 10 per cent yeast.

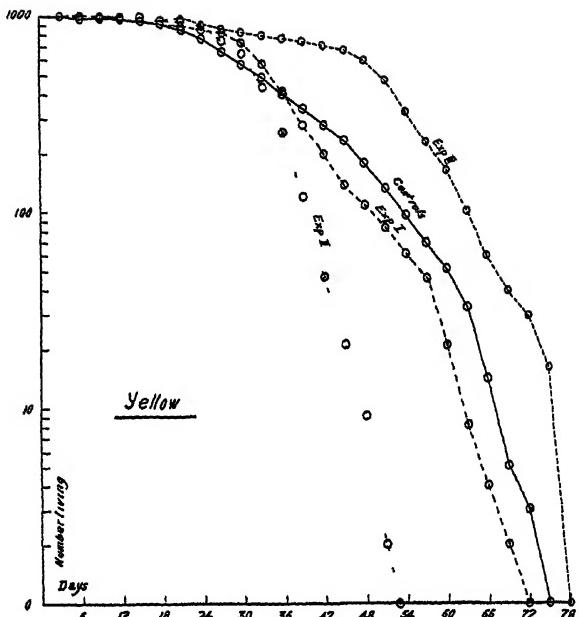


Fig. 7. Yellow. Life-curves of imagos.

Controls, first and second sets together.

Experiment I, flies aseptic as larvae and pupae, imagos run in same manner as controls.

Experiment II, flies aseptic throughout life; larvae on banana agar with 10 per cent yeast, imagos on banana agar without yeast.

Experiment III, flies aseptic throughout larval and imaginal life, on banana agar with 10 per cent yeast.

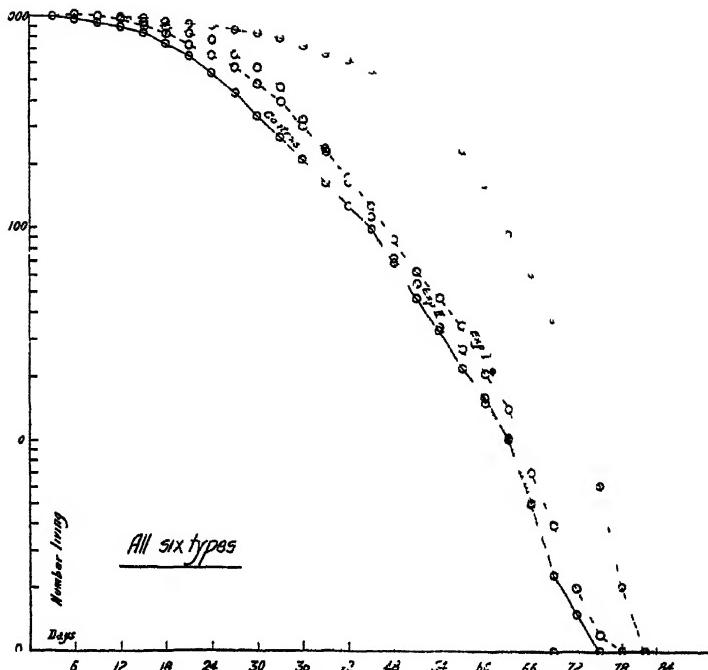


Fig. 8. All six types. Life-curves of imagoes.

Controls, first and second sets together.

Experiment I, flies aseptic as larvae and pupae, imagoes run in same manner as controls.

Experiment II, flies aseptic throughout life; larvae on banana agar with 10 per cent yeast, imagoes on banana agar without yeast.

Experiment III, flies aseptic throughout larval and imaginal life, on banana agar with 10 per cent yeast.

TABLE 1—FIRST SET OF CONTROLS
SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN DAYS, FOR SIX TYPES OF DROSOPHILA

Type	Number both sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma
Cinnabar	714	25.53±.20	11.61±.21	367	29.99±.47	13.49±.33	347	20.81±.23	6.43±.16
Curled	925	20.98±.20	9.01±.14	470	22.77±.44	14.24±.31	446	19.07±.24	7.47±.17
Vestigial	2,083	16.88±.12	7.98±.08	1,044	14.82±.16	7.42±.10	1,039	8.01±.17	8.01±.17
White	1,067	24.14±.14	8.42±.10	784	23.03±.21	8.79±.15	883	25.13±.18	7.84±.13
Wild	1,750	22.55±.15	9.17±.10	819	23.17±.23	9.89±.16	631	22.00±.19	8.64±.14
Yellow	1,322	26.28±.17	9.27±.12	595	23.38±.22	7.98±.16	727	28.65±.24	9.57±.17
All six types	8,461	22.13±.07	9.01±.06	4,088	21.61±.11	10.25±.08	4,373	22.02±.09	8.04±.06
All six types unweighted mean	22.73			22.86			22.35		

TABLE 2—SECOND SET OF CONTROLS
SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN DAYS, FOR SIX TYPES OF DROSOPHILA

Type	Number both sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma
Cinnabar	767	33.62±.34	14.10±.25	366	38.51±.56	16.87±.40	391	28.85±.35	10.20±.25
Curled	882	33.87±.31	13.48±.23	426	35.26±.48	14.50±.34	426	30.49±.39	11.88±.27
Vestigial	892	19.80±.28	12.36±.20	393	14.19±.32	9.41±.23	409	24.21±.38	12.63±.27
White	700	20.94±.31	12.20±.23	312	32.62±.51	13.29±.36	387	27.02±.37	11.04±.26
Wild	759	33.80±.24	10.01±.17	355	34.95±.37	10.20±.26	404	32.97±.32	0.65±.23
Yellow	1,402	40.40±.25	13.70±.18	616	38.20±.35	12.16±.23	786	42.12±.35	14.73±.25
All six types	5,371	32.51±.13	14.50±.09	2,468	32.73±.19	14.09±.14	2,903	32.33±.17	13.87±.12
All six types unweighted mean	31.74			32.27			31.09		

TABLE 3—FIRST AND SECOND SET OF CONTROLS. WEIGHTED MEANS
SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN DAYS, FOR SIX TYPES OF DROSOPHILA

Type	Number both sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma
Cinnabar	1,471	29.04±.24	13.67±.17	733	34.24±.38	16.30±.27	738	25.07±.24	9.52±.17
Curled	1,777	26.68±.21	12.83±.15	905	28.65±.31	13.81±.22	872	24.65±.26	11.39±.18
Vestigial	2,976	18.34±.12	9.50±.08	1,437	14.51±.14	8.01±.10	1,538	21.58±.17	10.13±.12
White	2,376	25.87±.14	10.07±.10	1,036	27.73±.23	11.18±.16	1,280	26.00±.17	9.06±.12
Wild	2,569	10.83±.16	1.174	25.26±.19	9.50±.13	1,325	25.32±.19	10.38±.13	
Yellow	2,724	33.65±.18	13.76±.13	1,211	30.92±.25	12.70±.17	1,513	35.64±.25	14.21±.17
All six types	13,832	26.16±.07	12.80±.05	6,556	25.79±.11	13.48±.08	7,276	26.50±.10	12.14±.07

TABLE 4

FIRST AND SECOND SETS OF CONTROLS. UNWEIGHTED MEANS

SHOWING MEANS OF DURATION OF LIFE OF IMAGOS IN DAYS, FOR SIX TYPES OF
DROSOPHILA

Types	Both sexes	Males	Females
Cinnabar	29.52	34.25	24.83
Curled	26.63	29.01	24.78
Vestigial	18.34	14.51	21.58
White.	27.04	27.78	24.96
Wild	28.22	29.06	30.81
Yellow .	33.34	30.79	35.38
All six types	27.32	27.56	27.06

TABLE 5
 EXPERIMENT I. ASEPTIC AS LARVAE AND PUPAE
 SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN
 DAYS FOR SIX TYPES OF DROSOPHILA

Type	Number held, sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma
Chumbar.....	668	32.05±.34	12.85±.24	301	38.07±.58	14.90±.41	307	28.25±.20	8.34±.21
Curled.....	714	26.86±.26	10.34±.18	342	26.51±.36	0.91±.26	372	27.19±.38	10.72±.27
Vestigial.....	692	23.75±.30	10.08±.21	323	10.33±.27	7.23±.19	269	28.96±.46	11.75±.34
White.....	628	33.59±.38	14.16±.27	263	33.05±.64	15.33±.46	305	33.33±.28	7.93±.20
Wild.....	526	28.32±.30	12.40±.26	244	26.34±.56	13.05±.40	282	30.80±.45	11.10±.32
Yellow.....	536	33.64±.33	11.34±.23	249	20.63±.43	29.90±.30	287	37.13±.44	11.13±.31
All six types, weighted means	3,694	29.81±.14	12.61±.10	1,732	28.71±.22	12.54±.16	1,942	30.70±.18	11.64±.13
All six types, unweighted means	20.84	30.06

TABLE 6

EXPERIMENT II. ASEPTIC ON BANANA AGAR WITHOUT YEAST
 SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN
 DAYS, FOR SIX TYPES OF DROSOPHILA

Variety	Number	Mean	Sigma
Cinnabar.....	528	26.31±.29	9.76±.20
Curled.....	243	27.43±.50	11.54±.35
Vestigial.....	619	27.10±.25	9.17±.18
White.....	483	35.29±.34	11.11±.24
Wild.....	500	40.46±.35	11.51±.25
Yellow.....	539	30.75±.12	7.27±.18
All six types—weighted mean.....	2,912	31.31±.14	11.23±.10
Unweighted mean.....		31.22

TABLE 7
EXPERIMENT III. ASEPTIC ON BANANA AGAR WITH YEAST
SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN DAYS, FOR SIX TYPES OF DROSOPHILA.

Type	Number both sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma
Cinnabar.....	961	43.02±.34	15.47±.24	527	46.22±.54	18.29±.38	434	41.19±.34	10.56±.24
Curled.....	844	39.74±.39	16.63±.27	290	37.1±.06	10.62±.47	410	41.87±.61	15.20±.36
Vestigial.....	711	40.56±.38	14.86±.28	427	42.0±.47	16.35±.36	284	38.35±.48	11.93±.34
White.....	654	48.34±.39	14.78±.28	337	61.0±.61	13.70±.36	317	45.61±.58	15.21±.41
Wild.....	810	47.82±.29	12.27±.21	406	47.44±.46	13.09±.32	404	49.20±.36	10.68±.25
Yellow.....	688	45.65±.40	15.47±.28	437	44.31±.55	17.16±.39	251	47.90±.50	11.04±.35
All six types, weighted mean.....	4,688	44.20±.16	15.35±.11	2,424	44.01±.23	16.71±.16	2,100	43.74±.19	13.16±.14
Unweighted mean.....	44.32	44.38	44.01

TABLE 8
CINNABAR
SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN DAYS, UNDER CONTROL AND EXPERIMENTAL CONDITIONS

Experiment	Number both sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma
First set of controls.....	714	25.53±.20	11.61±.21	367	29.90±.47	13.43±.33	347	20.81±.23	6.43±.16
Second set of controls.....	767	33.52±.34	14.10±.26	366	38.51±.66	15.87±.40	391	28.85±.36	10.20±.26
First and second sets of controls.....	1,471	29.64±.24	13.57±.17	733	34.24±.38	15.30±.27	738	25.07±.24	9.52±.17
Exper. I.....	688	32.95±.34	12.86±.24	301	38.07±.68	14.90±.41	367	28.25±.29	8.34±.21
Exper. II.....	528	26.31±.20	9.76±.20
Exper. III.....	961	43.92±.34	15.47±.24	627	46.22±.54	18.28±.38	434	41.12±.34	10.56±.24

TABLE 9

CURLED
SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN DAYS, UNDER CONTROL AND EXPERIMENTAL CONDITIONS

Experiment	Number both sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma
First set of controls.....	925	20.98±.20	9.01±.14	479	22.77±.44	14.24±.31	440	19.07±.24	7.47±.17
Second set of controls.....	852	22.87±.31	13.48±.22	426	35.26±.48	14.56±.34	426	30.49±.39	11.86±.27
First and second sets of controls.....	1,777	24.08±.21	12.83±.16	905	28.85±.31	13.8±.22	872	24.66±.26	11.59±.18
Exper. I.....	243	26.86±.26	10.34±.18	342	26.51±.36	1.91±.26	372	27.19±.38	10.72±.27
Exper. II.....	243	27.43±.50	11.54±.35	290	37.1 ±.66	10.62±.47	410	41.87±.51	15.26±.36
Exper. III.....	844	39.74±.39	16.63±.27						

TABLE 10

VESTIGIAL
SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN DAYS, UNDER CONTROL AND EXPERIMENTAL CONDITIONS

Experiment	Number both sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma
First set of controls.....	2,083	16.38±.12	7.99±.08	1,044	14.82±.16	7.42±.16	1,039	18.06±.17	8.01±.18
Second set of controls.....	892	19.80±.28	12.36±.20	393	14.19±.32	9.41±.23	499	24.21±.38	12.63±.27
First and second sets of controls.....	2,975	18.34±.12	9.60±.08	1,437	14.61±.14	8.01±.10	1,638	21.68±.17	10.13±.12
Exper. I.....	592	23.7 ±.30	10.69±.21	323	19.33±.27	7.23±.10	260	28.06±.48	11.75±.34
Exper. II.....	619	27.10±.25	9.17±.18	427	42.0 ±.47	16.35±.38	284	38.35±.48	11.93±.34
Exper. III.....	711	40.55±.38	14.36±.28						

TABLE 11
WHITE

SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN DAYS, UNDER CONTROL AND EXPERIMENTAL CONDITIONS

Experiment	Number both sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma
First set of controls.....	1,687	24.14±.14	8.42±.10	794	23.03±.21	8.76±.16	833	25.13±.18	7.94±.13
Second set of controls.....	709	29.94±.31	12.29±.23	312	32.63±.51	13.29±.36	397	27.92±.37	11.04±.26
First and second sets of controls.....	2,376	25.87±.14	10.07±.10	1,096	25.73±.23	11.13±.16	1,280	26.00±.17	9.06±.12
Exper. I.....	628	33.59±.38	14.16±.27	283	33.98±.64	15.33±.45	365	33.33±.28	7.93±.20
Exper. II.....	463	36.20±.34	11.11±.24	337	36.0±.51	13.76±.36	317	45.51±.53	15.21±.41
Exper. III.....	654	48.34±.39	14.78±.28						

TABLE 12
WILD

SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN DAYS, UNDER CONTROL AND EXPERIMENTAL CONDITIONS

Experiment	Number both sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma
First set of controls.....	1,760	22.55±.15	9.17±.10	819	23.17±.23	9.89±.16	931	22.00±.19	8.64±.14
Second set of controls.....	759	33.88±.24	10.01±.17	355	34.98±.37	10.20±.26	404	32.97±.32	9.65±.23
First and second sets of controls.....	2,509	25.98±.15	10.83±.10	1,174	26.73±.19	9.50±.13	1,335	25.32±.19	10.38±.13
Exper. I.....	526	28.32±.36	12.40±.26	244	25.34±.56	13.05±.40	282	30.89±.46	11.19±.22
Exper. II.....	600	40.49±.35	11.51±.25	405	47.44±.46	13.68±.32	404	49.20±.36	10.66±.25
Exper. III.....	810	47.82±.29	12.27±.21						

TABLE 13

YELLOW

SHOWING MEANS AND STANDARD DEVIATIONS OF DURATION OF LIFE OF IMAGOS IN DAYS, UNDER CONTROL AND EXPERIMENTAL CONDITIONS

Experiment	Number both sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma
First set of controls.....	1,322	26.28±.17	0.27±.12	505	27.30±.22	7.98±.10	727	28.05±.24	0.57±.17
Second set of controls.....	1,402	40.40±.25	13.70±.18	616	38.20±.33	12.10±.23	786	42.12±.36	14.73±.25
First and second sets of controls.....	2,724	33.55±.18	13.76±.13	1,211	30.92±.25	12.7±.17	1,513	35.04±.25	14.21±.17
Exper. I.....	630	33.64±.33	11.34±.23	249	29.63±.43	9.09±.30	287	37.13±.44	11.13±.31
Exper. II.....	510	30.75±.12	7.27±.18
Exper. III.....	638	45.65±.40	15.47±.28	437	41.31±.55	17.16±.39	351	47.09±.60	11.14±.36

TABLE 14
SUMMARY OF ALL SIX TYPES
SHOWING MEANS AND STANDARD DEVIATIONS ON DURATION OF LIFE OF IMAGOS IN DAYS, UNDER CONTROL AND EXPERIMENTAL CONDITIONS

Experiment	Number both sexes	Mean	Sigma	Number males	Mean	Sigma	Number females	Mean	Sigma	Sigma
First set of controls	8,461	22.13±.07	9.61±.05	4,088	21.61±.11	10.25±.08	4,373	22.02±.09	8.94±.06	
Second set of controls	5,371	32.61±.13	14.60±.09	2,408	32.73±.19	14.69±.14	2,963	32.33±.17	13.87±.12	
First and second sets of controls	13,832	26.10±.07	12.80±.05	6,550	25.79±.11	13.48±.08	7,276	26.50±.10	12.14±.07	
Exper. I	3,664	29.81±.14	12.61±.10	1,722	28.71±.22	12.54±.16	1,942	30.70±.18	11.64±.13	
Exper. II	2,912	31.31±.14	11.23±.10	2,424	44.91±.23	16.71±.16	2,100	43.74±.19	13.16±.14	
Exper. III	4,668	44.20±.15	15.35±.11							

Experiment	Both sexes	Males	Females	(b) UNWEIGHTED MEAN		
				Exper.	Exper.	Exper.
First set of controls	22.73	22.86	22.35			
Second set of controls	31.74	32.27	31.09			
First and second sets of controls	27.32	27.67	27.06			
Exper. I	29.84	28.9	30.96			
Exper. II	31.22	44.68	44.01			
Exper. III	44.32					

TABLE 15

FIRST SET OF CONTROLS

SURVIVORS ON DAYS INDICATED PER 1,000 FLIES STARTING IMAGINAL LIFE
THE SAME DAY

Age in days	Cinnabar	Curled	Vestigial	White	Wild	Yellow	All six types
1	1000	1000	1000	1000	1000	1000	1000
3	1000	1000	993	1000	1000	999	998
6	992	973	957	987	962	989	974
9	979	928	870	970	919	979	932
12	964	866	717	938	871	969	869
15	905	791	544	873	807	919	780
18	762	671	406	797	731	843	678
21	623	548	306	679	599	744	562
24	458	317	218	545	450	586	419
27	329	178	147	364	311	427	287
30	222	101	80	224	206	315	186
33	187	75	41	134	133	239	124
36	170	57	16	81	73	154	80
39	146	48	7	45	38	97	52
42	128	38	2	30	26	60	37
45	110	33	1	17	17	39	27
48	77	18	0	9	13	28	18
51	52	13		6	6	20	12
54	37	10		4	3	12	8
57	17	4		2	2	4	4
60	12	2		1	2	1	3
63	5	1		1	0	0	1
66	0	0		0			0

TABLE 16

SECOND SET OF CONTROLS

SURVIVORS ON DAYS INDICATED PER 1,000 FLIES STARTING IMAGINAL LINES
THE SAME DAY

Age in days	Cinnabar	Curled	Vestigial	White	Wild	Yellow	All six types
1	1000	1000	1000	1000	1000	1000	1000
3	999	1000	997	1000	1000	999	999
6	995	980	879	986	992	990	970
9	982	954	787	983	983	981	942
12	970	928	697	918	971	975	813
15	934	894	595	886	980	986	877
18	888	849	491	843	947	952	835
21	798	798	383	799	912	934	789
24	734	762	333	726	863	897	732
27	653	682	295	632	788	850	666
30	557	604	234	507	696	778	581
33	476	534	193	378	577	710	504
36	400	467	146	294	443	625	418
39	351	379	99	227	327	545	345
42	287	268	63	172	215	471	270
45	225	194	33	124	144	407	311
48	170	128	16	75	83	315	154
51	128	72	6	53	40	232	104
54	92	51	2	51	15	171	73
57	63	38	0	27	6	128	53
60	50	29		20	2	96	40
63	36	15		9	0	45	25
66	22	6		5		26	12
69	13	3		3		10	6
72	5	0		2		5	3
75	0			0		3	1
78						0	0

TABLE 17

FIRST AND SECOND SETS OF CONTROLS

SURVIVORS ON DAYS INDICATED PER 1,000 FLIES STARTING IMAGINAL LIFE
THE SAME DAY

Age in days	Cinnabar	Curled	Vestigial	White	Wild	Yellow	All six types
1	1000	1000	1000	1000	1000	1000	1000
3	999	1000	994	1000	1000	999	998
6	993	976	934	987	971	989	972
9	980	935	845	988	939	980	936
12	967	896	711	932	902	972	886
15	920	841	559	877	854	943	817
18	827	756	431	811	797	899	737
21	713	668	321	711	694	842	646
24	600	530	253	599	575	746	540
27	496	420	191	444	455	644	433
30	401	342	126	308	354	552	339
33	342	295	86	207	267	477	270
36	288	254	55	142	185	396	211
39	252	207	34	99	125	327	165
42	209	148	20	72	83	271	127
45	167	110	10	49	56	229	98
48	125	71	5	28	34	175	69
51	91	41	2	20	17	129	47
54	65	29	1	12	6	94	33
57	41	20	0	9	3	68	22
60	31	15		7	2	50	16
63	21	7		3	0	32	10
66	12	3		1		14	5
69	7	2		1		5	2
72	3	0		4		3	1
75	0			4		1	
78				0		0	0

TABLE 18

EXPERIMENT I. ASEPTIC AS LARVAE AND PUPAE

SURVIVORS ON DAYS INDICATED PER 1,000 FLIES STARTING IMAGINAL LIFE
THE SAME DAY

Age in days	Cinnabar	Curled	Vestigial	White	Wild	Yellow	All six types
1	1000	1000	1000	1000	1000	1000	1000
3	1000	997	997	998	1000	1000	999
6	1000	989	990	982	998	1000	993
9	997	982	978	987	996	998	989
12	985	957	938	981	960	954	964
15	967	898	823	963	751	924	892
18	928	794	677	920	698	896	822
21	862	668	498	817	673	879	733
24	760	556	397	728	658	830	652
27	605	489	314	643	587	802	568
30	484	397	259	564	483	711	477
33	427	301	221	462	409	556	391
36	365	201	159	371	329	399	301
39	302	152	112	301	251	273	230
42	253	103	81	252	167	194	175
45	208	52	52	214	95	135	126
48	163	21	31	163	50	107	89
51	118	7	15	132	21	82	63
54	85	5	7	112	10	60	47
57	60	3	5	88	6	45	35
60	34	2	3	67	2	21	22
63	24	2	0	45	2	8	14
66	10	2		24	0	4	7
69	2	0		16		4	4
72	0			15			2
75				8			1
78				2			0 3
81				2			0 3
84							0

TABLE 19

EXPERIMENT II. ASEPTIC ON BANANA AGAR WITHOUT YEAST
 SURVIVORS ON DAYS INDICATED PER 1,000 FLIES STARTING IMAGINAL LIFE
 THE SAME DAY

Age in days	Cinnabar	Curled	Vestigial	White	Wild	Yellow	All six types
1	1000	1000	1000	1000	1000	1000	1000
3	1000	996	1000	1000	1000	1000	1000
6	1000	988	995	1000	998	1000	997
9	1000	979	985	994	996	996	998
12	972	938	955	979	984	991	972
15	900	889	890	963	984	974	935
18	797	840	818	925	974	939	883
21	661	761	755	901	942	915	824
24	591	605	666	861	922	872	762
27	493	436	541	785	876	735	657
30	409	325	435	708	854	617	571
33	248	222	330	642	798	441	458
36	137	210	136	523	724	248	327
39	72	177	81	396	806	115	285
42	38	140	63	302	428	46	163
45	31	124	34	195	316	21	113
48	27	74	15	122	214	9	72
51	25	45	2	87	188	2	55
54	21	25	0	42	126	0	34
57	19	8		33	110		27
60	16	8		25	60		15
63	18	8		17	36		10
66	6	8		6	16		5
69	4	0		0	0		1
72	0						0

TABLE 20

EXPERIMENT III. ASEPTIC ON BANANA AGAR WITH YEAST
 SURVIVORS ON DAYS INDICATED PER 1,000 FLIES STARTING IMAGINAL LIFE
 THE SAME DAY

Age in days	Cinnabar	Curled	Vestigial	White	Wild	Yellow	All adults per
1	1000	1000	1000	1000	1000	1000	1000
3	1000	1000	1000	1000	1000	1000	1000
6	999	999	990	997	1000	997	997
9	995	989	984	997	994	987	991
12	981	962	966	988	988	977	977
15	966	923	948	974	974	962	957
18	952	860	923	956	964	942	932
21	934	820	886	930	958	924	908
24	909	794	850	907	940	891	881
27	891	758	819	888	927	843	855
30	863	712	779	856	914	801	822
33	776	660	727	829	893	767	774
36	656	594	647	791	808	750	713
39	585	533	560	775	831	717	660
42	517	488	482	751	795	682	612
45	453	428	399	677	730	647	548
48	400	373	347	619	580	577	475
51	348	325	293	555	427	455	394
54	303	254	214	434	321	313	306
57	237	194	152	350	246	220	231
60	192	132	87	236	158	158	160
63	133	72	52	135	67	99	93
66	103	39	32	83	36	68	61
69	72	20	22	34	19	49	37
72	19	8	12	15	11	29	18
75	8	1	4	5	4	16	6
78	5	0	0	0	0	1	1
81	1	—	—	—	—	0	0 2
84	0	—	—	—	—	—	—

ON THE MORPHOLOGY OF PYRSONYMPHA
WITH A DESCRIPTION OF THREE NEW
SPECIES FROM RETICULITERMES
HESPERUS BANKS

BY

WILLIAM NOTTINGHAM POWELL

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY
Volume 31, No. 10, pp. 179-200, plates 9-11, 4 figures in text
Issued March 2, 1928

UNIVERSITY OF CALIFORNIA PRESS
BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS
LONDON, ENGLAND

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INTRODUCTORY AND HISTORICAL

This paper deals with the morphology of three species of *Pyrsonympha*, a flagellate from the intestine of *Reticulitermes hesperus* Banks, a termite found abundantly in the San Francisco bay region of California. Division stages of one of the species were found and a description of this process comprises a portion of the paper.

The genus *Pyrsonympha* was established by Leidy in 1877. He described *Pyrsonympha vertens* from *Termes flavipes* (now known as *Reticulitermes flavipes*), a termite widely distributed throughout the eastern part of the United States. Leidy classed *Pyrsonympha vertens* as an infusorian. Grassi and Sandias (1893) working with Italian termites described another species, *Pyrsonympha flagellata*. Later, however, Grassi (1911, 1917) considered his earlier classification as erroneous and referred this form to the genus *Spirotrichonympha*, as *Spirotrichonympha flagellata*. Porter (1897) redescribed Leidy's species, *Pyrsonympha vertens*. Comes (1910) described what was undoubtedly a species of *Pyrsonympha* from *Termes lucifugus* (now

known as *Reticulitermes lucifugus* Rossi) under the name of *Lophophora vacuolata*. Later (1912), he described as *Pyrsonympha flagellata* organisms which really belong to *Spirotrichonympha* and *Holomastigotes*. Zulueta (1915) described *Dinenymphia gracilis* from *Reticulitermes lucifugus* but his organism was without doubt a species of *Pyrsonympha*. Koidzumi (1921) described two species, *Pyrsonympha grandis* and *Pyrsonympha modesta* from *Leucotermes speratus* (= *Reticulitermes speratus* Kolbe) and *Leucotermes flaviceps* (= *Reticulitermes flaviceps* Oshima), termites from Japan and Formosa, respectively.

Pyrsonympha belongs in the order *Polymastigina*.

I am very much indebted to Dr. C. A. Kofoid, under whom this work was done, for his helpful advice and criticism.

MATERIAL AND TECHNIQUE

All the termites used in this investigation were procured from the Golden Gate Park in San Francisco.

Before intestinal smears were made from the termites they were placed in moist chambers on a filter paper diet for several days. As a result of this procedure much cleaner smears can be obtained; in addition, the change from wood to filter paper diet seems to stimulate division of the protozoa, as was suggested by Kirby (1926).

Smears were made by teasing out the intestines of the termites into 0.4 per cent salt solution on slides. The slides were coated very thinly with albumen fixative beforehand and only a small amount of salt solution was used else there was a great loss of the organisms when the slides were plunged into the fixing agent. A number of fixing agents were employed, among them being Schaudinn's sublimate-alcohol, Bouin's fluid, Zenker's fluid, Flemming's without acetic, Champy's fluid, and osmic vapor. The best general results were obtained with fixation in Bouin's fluid for twenty minutes at 50–55° C. The preparations were stained in Heidenhain's iron-alum haematoxylin, phosphotungstic acid haematoxylin, and Regaud's iron-alum haematoxylin. Of these stains Heidenhain's iron-alum haematoxylin with a light counterstain of eosin gave the best general results. Regaud's iron-alum haematoxylin, after fixation in osmic vapor, proved useful for the demonstration of mitochondria.

Several intestines were fixed whole in Bouin's fluid, imbedded, sectioned at 10 microns, and stained in Heidenhain's iron-aum hae-

matoxylin. Such preparations were less useful for general morphological observation but were of value in showing the mode of attachment of the flagellates to the intestinal wall of the termite.

Observations of living material were made in 0.4 per cent salt solution.

GENERAL MORPHOLOGY

Several forms of *Pyrsonympha* are present in the intestine of *Reticulitermes hesperus*. After considerable study the conclusion has been reached that these represent three species and they are described accordingly.

Pyrsonympha occurs both free in the lumen of the intestine and attached to the intestinal wall. The free-swimming forms do not move about actively although they keep their flagellar cords in constant motion and swing their anterior ends about actively. In addition, the axostyle is often vigorously lashed about in the endoplasm. I have not observed the method by which these flagellates ingest wood but Porter (1897) figures a *Pyrsonympha* apparently taking in a fragment of wood at the rounded posterior end of the body. This agrees with the location of ingestion described for *Trichonympha campanula* by Swezy (1923) and by Cleveland (1925).

Pyrsonympha minor sp. nov.

Plate 9, figures 1, 2

Pyrsonympha minor sp. nov. is the smallest species of *Pyrsonympha* found in *Reticulitermes hesperus*. In length it measures from 30 to 85 microns, in width or diameter from 20 to 50 microns. The average length is 60 microns, the average width 30 microns. The ratio of the average length to the average width is 2:1. The posterior two-thirds of the body is usually fairly uniform in width; the point of least diameter is usually situated about one-third of the total length from the anterior tip of the body. The anterior third is generally slightly tapered toward the tip although in many cases the extreme anterior end is rather broadly rounded. On the whole, although there is considerable variation, the body is distinctly club-shaped. In outline it is usually somewhat serrated; this appearance is due to the ridges along which run the flagellar cords. The body is often slightly twisted, as is the case in some species of *Dinenympha*. The surface

is free from attached bacteria. In shape and size this species closely resembles *Dinenymphia gracilis* (really a *Pyrsonymphia*) of Zulueta (1915) and *Pyrsonymphia modesta* of Koidzumi (1921).

The endoplasm of *Pyrsonympha minor* is vacuolated, the range in the size of the vacuoles or alveoles being well represented in the specimens figured (pl. 9, figs. 1, 2). In some cases small granules are visible at the margins of the vacuoles. In many specimens what are presumably metabolic granules are present. Under normal conditions the endoplasm, is, of course, more or less filled with fragments of wood.

The axostyle of *Pyrsonympha minor* is attached at its anterior end to a short, rod-like centroblepharoplast; posteriorly, the axostyle is free in the endoplasm extending to the posterior tip of the body in most cases. The axostyle is homogeneous in appearance and stains deeply in iron-alum haematoxylin. It is comparatively slender and tapers fairly uniformly from the anterior to the posterior end. In the great majority of individuals the posterior one-third or two-thirds of the axostyle is split into two portions, in a few cases into three or more portions. Sometimes, indeed, the axostyle is split in this fashion along almost its entire length.

The centroblepharoplast is located at the extreme anterior point of the body. It is a short, rod-like structure terminating anteriorly in a triangular, deeply staining granule. To it are attached the axostyle, the nucleus, and the flagellar cords. In addition to its function as a neuromotor center it also seems to serve as the means of attachment of the organisms to the intestinal wall (pl. 10, fig. 7).

The flagellar cords apparently arise in succession along the length of the centroblepharoplast. They run posteriorly as an evenly spaced group in a leiotropic spiral about the body, terminating posteriorly as free flagella. The forms with eight flagellar cords predominate although some with four cords are seen. Undoubtedly, the former represent adult individuals, the latter, individuals recently divided. The doubling of the flagellar cords evidently occurs very soon after division since many quite small specimens are of the eight-cord type. In most cases the flagellar cords run on the edges of distinct ridges thus giving the body a serrated transsection. The flagellar cords generally make one complete turn about the body.

The integration of the neuromotor elements is usually difficult to determine. The point of attachment of the nucleus to the centroblepharoplast seems to be slightly behind that of the axostyle. The

slender, thread-like connection between the nucleus and the centroblepharoplast may be called the rhizoplast (pl. 9, fig. 2).

The nucleus is always close up behind and attached to the centroblepharoplast. In shape, the nucleus is almost spherical, sometimes slightly pyriform. There is a slight amount of elongation at the region of attachment of the rhizoplast, which region is often darkly stained. There is a distinct nuclear membrane. Internally the nucleus exhibits finely granular chromatin imbedded in a faintly staining linin network. A single endosome, of variable size and shape, surrounded by a halo, is always present in the posterior part of the nucleus; in addition there are several or more considerably smaller dark bodies scattered through the nucleus.

Pyrsonympha granulata sp. nov.

Plate 9, figures 3, 4

Pyrsonympha granulata sp. nov. is typically club-shaped, being broad posteriorly and tapering more or less uniformly toward the narrower anterior end. A very great variation in form is exhibited, however, due apparently to the contractility of the body. Both long, slender and short, stout, forms are found. The posterior end of the body is nearly always greatest in diameter but some greatly elongated specimens are practically uniform in diameter throughout their entire length and in a few cases the middle third of the body is greatest in diameter. The body is generally least in diameter just back of the nucleus but this is not always the case; it is usually irregular in contour but this feature also is variable. The surface of the body is free from attached bacteria. This species varies in length from 40 to 120 microns and in width or diameter from 5 to 35 microns. The average length is 83 microns, the average width 16 microns. The ratio of the average length to the average width is 5.2:1.

Pyrsonympha granulata owes its specific name to the fact that the endoplasm practically always contains a number of densely staining granules. These granules occur in varying numbers, sometimes almost completely filling the body, in other cases being rather sparsely distributed. There seems to be no particular rule as regards their distribution although they are most often numerous in the middle or posterior third of the body. They are seldom found anterior to or along the sides of the nucleus; the reason for this is obvious since the nucleus always occupies most of the available space in the endoplasm

at the extreme anterior end of the body. The granules show some variability in their staining properties ranging in color from dark gray to dense black. In shape they are always exactly spherical. In size they range from two microns down to the limits of microscopical vision. In a few cases the individual granules seem to be surrounded by halos. The nature of these granules is unknown; possibly they are metabolic products. The portion of the endoplasm which is not filled with the larger granules is very finely granular in appearance, seldom vacuolated. Under normal conditions, of course, more or less wood is present in the endoplasm.

The axostyle of this species is attached at the anterior end to the centroblepharoplast; posteriorly the axostyle hangs free in the endoplasm. The axostyle is homogeneous in appearance and stains intensely in iron-alum haematoxylin. It is largest in diameter at the attached anterior end and tapers more or less uniformly posteriorly. In the majority of specimens the axostyle is longer than the body and is bent back anteriorly at the extreme posterior end (pl. 9, fig. 3). The axostyle is usually single throughout its whole length but in large forms it is split into two or more portions for the posterior one-half or two-thirds of its length.

The centroblepharoplast is located at the anterior tip of the body. It is a short, rod-like structure ending anteriorly in a triangular granule. In some specimens this granule is prolonged into a short rod or thread which ends in a small knob. This knob apparently serves as an organ of attachment to the intestinal wall; in some specimens there can be seen attached to the knob irregular, faintly staining strands or fragments of what is presumably chitin from the intestinal wall of the termite.

The flagellar cords arise successively along the length of the centroblepharoplast. These run as an evenly spaced group in a leiotropic spiral about the body to end posteriorly as free flagella. The flagella cords usually make two and a half to three complete turns about the body, in some cases, however, only two complete turns. In most cases the cords lie on the edges of small ridges, although in some specimens they lie on the smooth surface of the body like so many tiny ropes. Both four- and eight-corded forms are present, the former predominating. Evidently the four-corded forms are recently divided individuals and the eight-corded forms are adults.

The integration of the neuromotor elements seems to be practically the same as in *Pyrsonymptha minor*.

In nearly all cases the nucleus is at the extreme anterior end of the body close behind and in contact with the centroblepharoplast. Sometimes, however, it is found slightly posterior to the centroblepharoplast and not visibly connected with it. The nucleus exhibits considerable variation in shape although it is usually pyriform. In all cases there is a greater or less degree of elongation at the region of its attachment to the centroblepharoplast; the connecting strand may be called a rhizoplast. Internally the nucleus contains finely granular chromatin arranged on a network of faintly staining linin threads. A single endosome is always present in the posterior part of the nucleus; this varies considerably in size and shape, being sometimes almost spherical, sometimes irregular in outline, sometimes rod- or wedge-shaped. In all cases it is surrounded by a distinct halo.

Pyrsonympha major sp. nov.

Plates 10, 11, figures 5-17

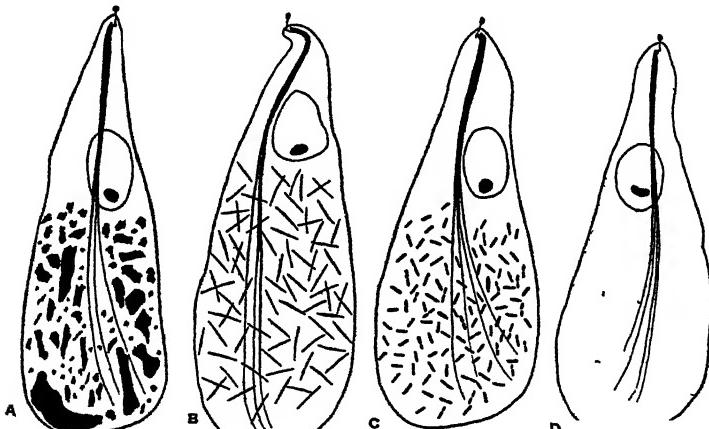
Pyrsonympha major sp. nov. is the largest species of *Pyrsonympha* found in *Reticulitermes hesperus* and the largest protozoan found in that termite. It varies greatly in size, however, some few specimens being no larger than forms of *Pyrsonympha minor*. In length *Pyrsonympha major* ranges from 75 to 140 microns, in width from 25 to 45 microns (pl. 10, figs. 8-10). The average length, computed from measurements of a number of individuals, is 106 microns, the average width 35 microns. The ratio of the average length to the average width is 3:1.

A comparison of the dimensions of some of the described species of *Pyrsonympha* might be of interest. Leidy (1881) gives 100-160 microns as the range in length of *Pyrsonympha vertens*. Porter (1897) states that he found specimens of *Pyrsonympha vertens* measuring up to 275 microns in length. Koidzumi (1921) gives 40-150 microns as the range in length and 19-40 microns as the range in width of his *Pyrsonympha grandis*. *Pyrsonympha major* is then of approximately the same dimensions as Koidzumi's species.

Pyrsonympha major is typically club-shaped, tapering uniformly from the rounded posterior end to the more or less pointed anterior end. As a rule the region of greatest width is at the extreme posterior end of the region of least width at the extreme anterior end of the body.

The endoplasm of *Pyrsonympha major* is sometimes finely granular in appearance, sometimes vacuolated. In general the larger

individuals are vacuolated, the smaller ones finely granular (pl. 10, figs. 8-10). In many cases granules very similar to those found in *Pyrsonymphia granulata* are present in *Pyrsonymphia major* but practically never in such great number as in that species. Individuals from termites feeding on their normal diet of wood are usually more or less filled with wood particles (fig. A). Sometimes bacteria of various sorts are also present in the endoplasm. Figures B and C represent two common types of bacterial inclusions. From the fact that these bacteria are not enclosed in vacuoles it seems that possibly they represent an infection, or even commensals. Individuals fixed in osmic vapor and stained in Regaud's iron-alum haematoxylin



Figs. A. B. C. D. Food inclusions of *Pyrsonymphia major* sp. nov. $\times 500$.

(Causey, 1925) have their endoplasm completely filled with tiny rod-like bodies which, from the constancy of their appearance under proper conditions of technique, are taken to be mitochondria (fig. D).

The axostyle of *Pyrsonymphia major* is attached at its anterior end to the centroblepharoplast. Posteriorly it ends freely in the endoplasm. In young individuals the axostyle is rather slender and stains deeply in iron-alum haematoxylin, being closely similar to the axostyles of *Pyrsonymphia minor* and *Pyrsonymphia granulata* (pl. 10, figs. 8, 10). In large individuals, however, the anterior end of the axostyle is thickened and does not as a rule stain homogeneously, it being possible in some cases to distinguish a deeply staining wall and less deeply staining internal portion. In all individuals of this species the axostyle is split into two, three, four, or more portions. Generally young individuals have axostyles split into two filaments, in the older forms the axostyles split into three or more filaments. The axostyle

evidently serves as a means of stirring up the endoplasm and its contents, and this splitting would tend to increase its effectiveness for this purpose.

At its anterior end the axostyle connects with the centroblepharoplast. The mode of connection varies somewhat. In some cases the axostyle is split at its anterior end into several strands all of which unite with the posterior granule of the centroblepharoplast. In other cases the axostyle is split into two portions, only the lower of which units with the centroblepharoplast. In many cases the anterior end of the axostyle stains faintly. Figure 9, plate 10, illustrates the two common modes of connection.

The centroblepharoplast is at the extreme anterior end of the body. It consists typically of a large anterior granule or knob connecting by a short, thick rod to a somewhat smaller posterior granule. The whole structure stains deeply in iron-alum haematoxylin. The centroblepharoplast serves as an organ of attachment as well as a neuromotor center. Figure 7, plate 10, is a semidiagrammatic representation of the method by which all three species of *Pyrsonympha* are attached to the intestinal wall. A short rod, or thread, projecting forward from the anterior knob of the centroblepharoplast, is imbedded apparently in the layer of chitin which lines the wall of the intestine. I have seen no evidence of a large peduncle such as is described in *Pyrsonympha vertens* by Porter (1897).

Practically all the individuals of this species possess eight flagellar cords (pl. 10, fig. 9). Very young, growing individuals, however, have four flagellar cords (pl. 10, fig. 8). This number is soon doubled by the outgrowth of four new cords alongside the original four (pl. 10, fig. 10). The flagellar cords arise from the centroblepharoplast and run posteriorly in a leiotropic spiral about the body to end as free flagella at the posterior end. As contrasted with the condition in *Pyrsonympha minor* and *Pyrsonympha granulata* the flagellar cords in *Pyrsonympha major* are not grouped but are equally spaced over the whole surface of the body. The flagellar cords usually make one complete turn about the body but in some cases they run almost parallel to the length of the body and show almost no spiraling. It is extremely difficult to make out the precise points of attachment of the flagellar cords to the centroblepharoplast. However, in one favorably stained and oriented specimen I have been able to determine this with a fair degree of certainty. The flagellar cords arise from, or extremely close to, the two end granules or knobs of the

centroblepharoplast (pl. 10, fig. 6). The flagellar cords of *Pyrsonympha major* are usually situated on ridges. This is much more evident in living than in stained material.

The nucleus of *Pyrsonympha major* is located a short distance back of the extreme anterior end of the body. Sometimes it may be as far back as the middle third of the body but this is exceptional. It is usually ovoidal but sometimes it is elongated anteriorly so as to be almost pyriform. The nucleus is attached to the posterior granule of the centroblepharoplast by a rhizoplast (pl. 10, fig. 5). This is to be seen only in a few individuals, however. Internally the nucleus exhibits a fine linin network in which are imbedded fine chromatin granules. A single endosome of variable shape is always present in the posterior end of the nucleus. It is surrounded by a distinct halo. In addition to the endosome there are usually several other similar smaller structures scattered through the nucleus. It is difficult to say whether or not they are of the same nature as the endosome.

NUCLEAR DIVISION

Division stages of *Pyrsonympha major* appear in several of my slides made from termites which had been fed for several days on filter paper. Although division stages are present in no great quantity, a sufficient number is present to indicate the main details of the process. They were distinguished as division stages of *Pyrsonympha major*, rather than of *Pyrsonympha minor* or *Pyrsonympha granulata*, by a comparison with the vegetative individuals on the same slides. With the exception of one individual (pl. 11, fig. 12) the specimens figured are not stages of division in the largest individuals of the species, suggesting a series of repeated binary fissions.

The body rounds up during the prophase, becoming amoeboid and irregular. Kirby (1924) states that the presence of wood in the endoplasm of *Dinenympha fimbriata* during division is one means of distinguishing it from *Pyrsonympha*. Since all my division stages are in specimens from termites fed on filter paper I cannot say whether or not wood is normally present in the endoplasm during division. In several of my division stages of *Pyrsonympha major* the endoplasm is distinctly alveolar or vacuolated but since vegetative individuals exhibit both granular and alveolar endoplasm this perhaps has no particular significance. Small, deeply staining granules

are present between the alveoles in several of my division stages (pl. 11, figs. 14, 15).

In the prophase the endosome or the nucleolus as it may be called from its behavior during division, in most cases breaks up into a number of smaller bodies which are scattered throughout the nucleus and gradually disappear. I have seen several prophase stages, however, in which the nucleolus seemed to be disappearing as a single body. As the nucleolus is disappearing the chromatin granules on the linin network become larger and more numerous. This gives the appearance of a number of heavy, knotty threads which stain very black in iron-alum haematoxylin. These threads, which are joined together by unstained linin threads, shorten and thicken and the connections between them disappear (pl. 11, fig. 11). At this time the threads are arranged peripherally in the nucleus. The process of shortening and thickening continues until V-shaped chromosomes are produced. The number of these is somewhere between twenty-five and thirty.

While the process of chromosome formation is going on, the centroblepharoplast divides longitudinally. Previous to this division the axostyle has been cast loose into the endoplasm where it is soon resorbed. With the division of the centroblepharoplast the flagellar cords are divided into two groups of four each, one group to each of the centroblepharoplasts. Very shortly after this division occurs, new axostyles begin to grow out from each of the centroblepharoplasts (pl. 11, fig. 12).

Rather heavy intranuclear spindle fibers appear in the later stages of the prophase. These stain rather deeply with iron-alum haematoxylin. The nuclear membrane remains intact up to the time of actual division of the nucleus.

The chromosomes become arranged in a mass on the equatorial plate in the metaphase stage of division (pl. 11, fig. 13). The spindle is very distinct and, except for the persistence of the nuclear membrane, has the appearance of a metazoan spindle. The centroblepharoplasts are at the poles. I was not able to determine at what stage splitting of the chromosomes occurs. Kirby (1924) states that in *Dinenynmppha fimbriata* splitting occurs in the prophase threads before the formation of definite chromosomes.

In the anaphase (pl. 11, figs. 14-16) the halves of the chromosomes separate and are drawn toward the poles. By this time the new axostyles are quite long. The chromosomes are distinct in one of my

anaphase stages (pl. 11, fig. 15) and a number of fibers run between the groups of chromosomes (pl. 11, fig. 15). A distinct paradesmose lying on top of the nuclear membrane, and connecting the two centroblepharoplasts, can be seen in plate 11, figure 16.

The nucleus constricts into two, leaving rather long points at the region of separation (pl. 11, fig. 17). Figure 17, plate 11, shows an abnormal division stage in which there are five flagellar cords at one pole and six at the other. Evidently this is a case of premature out-growth of the new cords since one flagellar cord at the pole having five, and two at the pole having six, are distinctly heavier at their bases than are the other four.

Plasmotomy probably occurs rapidly. The chromosomes in the nucleus of each new individual break down into heavy threads which at length form the typical reticulum of the resting nucleus. In figure 8, plate 10, is represented a recently divided individual in which the chromosomes have formed threads. The nucleolus has not yet reappeared. The manner in which it reappears could not be determined. In *Dinenymphia fimbriata* (Kirby, 1924) globular masses appear in the nucleus and collect to form the nucleolus.

DISCUSSION

The axostyle.—The axostyle in *Pyrsonymphidae* is attached at its anterior end to the centroblepharoplast; posteriorly it ends freely in the endoplasm. At division it is cast off into the endoplasm and resorbed; new axostyles grow out from the centroblepharoplasts. Leidy (1877) speaks of the axostyle of *Pyrsonympha vertens* as a narrow band running the length of the body. Again (1881) he calls it an undulating cord. Porter (1897) calls it a flagellum. He says that it is attached at its posterior end in *Pyrsonympha vertens* and figures it as projecting into a tail-like appendage at the posterior end of the body in some cases. Comes (1910) speaks of the axostyle of *Lophophora vacuolata* (= *Pyrsonymphidae*) as a "bastoncello scheletrico." This may be translated as "skeletal rod." Zulueta (1915) calls the structure in *Dinenymphia gracilis* (= *Pyrsonymphidae*) an "axostilo." Koidzumi (1921) employs the term "axial filament" instead of axostyle and states that he does not regard it as homologous with the axostyle of trichomonad flagellates since an axostyle is only skeletal in function. However, the axostyles of trichomonad

flagellates do serve as motor organs, as is the case in *Tritrichomonas augusta* (Kofoid and Swezy, 1915). Koidzumi further advances the idea that the "axial filament" of *Pyrsonympha* functions as a basal granule or blepharoplast for the flagellar cords. My preparations show, however, a definite centroblepharoplast in *Pyrsonympha* from which the flagellar cords arise and from which the axostyle itself grows out during division. In its stainability the axostyle of *Pyrsonympha* resembles that of *Trichomonas buccalis* (Hinshaw, 1926) and *Giardia enterica* (Kofoid and Swezy, 1922). I agree with Kirby (1924) in his use of the term axostyle and can see no reason for Koidzumi's conception of the "axial filament" as distinct from the axostyle.

The flagellar cords.—Leidy (1877) speaks of the flagellar cords of *Pyrsonympha vertens* as moving lines. In 1881 he calls them undulating lines. Porter (1897) calls them contractile or muscular cords and states that they do not end freely posteriorly but that each one can be traced to the posterior end of the body and then back again on the other side of the body to the anterior end. Comes (1910) and Zulueta (1915) refer to them as undulating membranes. Zulueta figures them rather well and shows them ending freely posteriorly. Koidzumi (1921) uses the term flagellar cords which is the term adopted by Kirby (1924) for his *Dinenymphia fimbriata*. The flagellar cords of *Pyrsonympha* possibly arose by the fusion of free flagella with the surface of the body since they lie only on the surface and are free at their posterior ends. Or they may possibly have arisen by inclusion in the surface layer or plasma-membrane on outgrowth from the centroblepharoplast. They are perhaps homologous with the marginal filament of the undulating membrane of *Trichomonas*.

The centroblepharoplast.—The centroblepharoplast of *Pyrsonympha* functions both as a blepharoplast from which arise the flagellar cords and axostyle, and as a division center, as in the case in *Trichonympha campanula* (Kofoid and Swezy, 1919). In addition the centroblepharoplast in *Pyrsonympha* functions as an organ of attachment. The granules at the ends of the centroblepharoplast in *Pyrsonympha major* may be homologized with the two blepharoplasts of *Pentatrichomonas ardin delteilii* (Kofoid and Swezy, 1923). In this flagellate there are two blepharoplasts, a posterior or primary blepharoplast and an anterior or secondary blepharoplast. From the former arise four flagella, from the latter one free flagellum, the undulating membrane, the parabasal, and the axostyle. In *Pyrso-*

nympha major, from the anterior granule of the centroblepharoplast arise four flagellar cords; from the posterior granule, four flagellar cords and the axostyle.

The paradesmose.—Hall (1923) distinguishes two types of paradesmose, the centrosome-paradesmose and the blepharoplast-paradesmose. The paradesmose in *Pyrsonymphia major* is a blepharoplast-paradesmose similar to that of *Trichonympha campanula* (Kofoid and Swezy, 1919).

Mitochondria.—Mitochondria have been reported from a number of flagellates. Alexeieff (1917) reports mitochondria from a number of flagellates among them being *Trypanosoma*, *Cryptobia*, *Trichomonas*, and *Tritrichomonas*. Later workers, however, have had some doubts as to the accuracy of Alexeieff's works since he did not employ a standardized technique. Causey (1925) reports mitochondria from *Leishmania braziliensis* and *Euglena gracilis*. Duboscq and Grassé (1925) describe mitochondria in *Pyrsonymphia vertens* but their findings do not agree with mine in regard to the number and distribution of the mitochondria. According to their account no mitochondria are found in the anterior end of the body while my preparations show mitochondria scattered throughout the whole of the endoplasm.

SUMMARY AND CONCLUSIONS

1. *Pyrsonymphia minor* sp. nov., *Pyrsonymphia granulata* sp. nov., and *Pyrsonymphia major* sp. nov. occur in the intestine of the termite *Reticulitermes hesperus* Banks both free in the lumen and attached to the intestinal wall.
2. The flagellar cords arise from the anterior centroblepharoplast and run backward in a leiotropic spiral about the body to end as free flagella posteriorly.
3. Young, recently divided forms have four flagellar cords; adult individuals, eight flagellar cords.
4. The nucleus contains a linin network in which are imbedded fine granules of chromatin and an endosome which is a true nucleolus since it disappears at mitosis without taking any part in chromosome formation.
5. Chromosomes are formed at division by the collection and concentration of chromatin along the linin threads. There is no continuous spireme.

6. The axostyle is cast off into the endoplasm and resorbed during division. New axostyles grow out from the centroblepharoplasts.

7. There is an achromatic spindle formed within the nuclear membrane which remains intact during division.

8. The centroblepharoplast divides longitudinally at the beginning of the mitotic process. The eight flagellar cords are divided into two groups of four each and one group remains attached to each centroblepharoplast at division. Soon after division four new flagellar cords grow out from the centroblepharoplast alongside the old flagellar cords thus giving rise to the adult number of eight.

9. *Pyrsonympha* seems to be related to the trichomonad flagellates in regard to the nature of the axostyle and the possible homology of the flagellar cords with the marginal filament of the undulating membrane in *Trichomonas*. It might be regarded then as a highly modified trichomonad. *Pyrsonympha* belongs in the order *Polymastigina*.

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EXPLANATION OF PLATES

EXPLANATION OF PLATES

All figures are drawn with camera lucida from material fixed in Bouin's fluid and stained in Heidenhain's iron alum haematoxylin. Magnification $\times 1000$.

PLATE 9

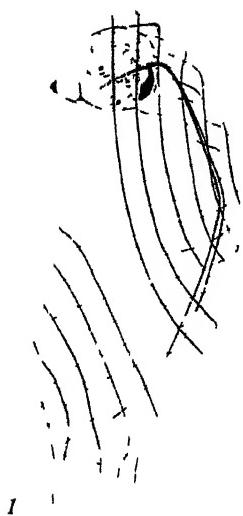
Species of *Pyrsonympha* from *Reticulitermes hisprous* Banks

Fig. 1. *Pyrsonympha minor* sp. nov. having finely vacuolated endoplasm.

Fig. 2. *Pyrsonympha minor* having coarsely vacuolated endoplasm. Note the rhizoplast connecting the nucleus to the centroblepharoplast.

Fig. 3. *Pyrsonympha granulata* sp. nov. having four flagellar cords. The shape of the posterior end of the body is evidently due to contraction. Attachment of nucleus and axostyle to centroblepharoplast is shown. The axostyle is not split.

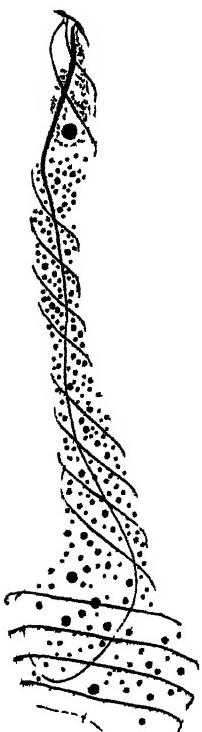
Fig. 4. *Pyrsonympha granulata* having eight flagellar cords. The axostyle is split into four portions.



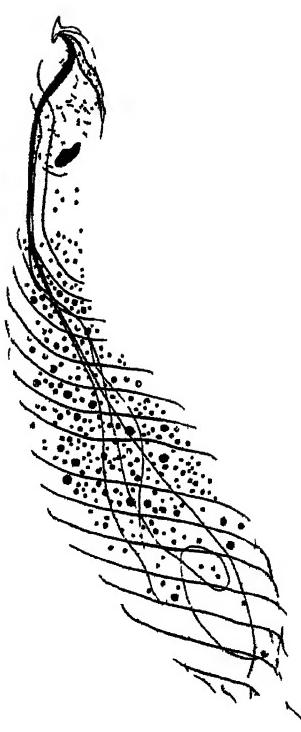
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PLATE 10

Vegetative individuals of *Pyrsonympha major* sp. nov.

Fig. 5. Semidiagrammatic figure showing the attachment of the nucleus to the centroblepharoplast by the rhizoplast.

Fig. 6. Shows connection of flagellar cords to centroblepharoplast.

Fig. 7. Mode of attachment to intestinal wall.

Fig. 8. Recently divided form with four flagellar cords. Nucleus has not yet gone back to resting condition.

Fig. 9. Large *Pyrsonympha major* having eight flagellar cords. Two common methods of attachment of the axostyle to the centroblepharoplast are shown. Axostyle is split into a number of filaments.

Fig. 10. Form of *Pyrsonympha major* intermediate between figures 8 and 9 showing outgrowth of new flagellar cords.



PLATE 11

Division stages of *Pyisonympha major*

Fig 11 Prophase Centriolepharoplast dividing, axostyle being cast off into endoplasm, body rounding up A number of short, deeply staining, disconnected threads are in the nucleus

Fig 12 Chromosomes formed in large individual Two centriolepharoplasts sending out new axostyles Flagellar cords could be seen only on the centriolepharoplast at the edge of the body

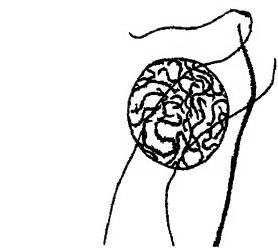
Fig 13 Metaphase Round, dark bodies are possibly fragments of the nucleolus which have not disappeared

Fig 14 Early anaphase Chromosomes separating Four flagellar cords attached to each centriolepharoplast

Fig 15 Anaphase showing distinct chromosomes Portion of old axostyle not yet resorbed by endoplasm

Fig 16 Anaphase Paradesmose connecting the centriolepharoplasts Fragments of old axostyles still present

Fig 17 Nuclear membrane constricting Axostyles fairly long and heavy Abnormal number of flagellar cords probably due to premature outgrowth The cords with heavy bases are taken to be new ones



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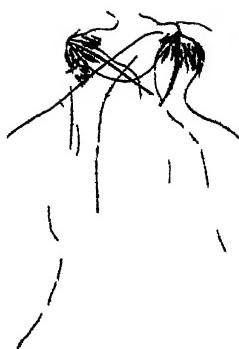
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A STUDY OF PHYSICAL AND CHEMICAL
CONDITIONS IN SAN FRANCISCO BAY
ESPECIALLY IN RELATION TO THE TIDES

BY

ROBERT C. MILLER, WILLIAM D. RAMAGE, AND EDGAR L. LAZIER

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY
Volume 31, No. 11, pp. 201-267, 5 figures in text and 5 charts
Issued May 8, 1928

UNIVERSITY OF CALIFORNIA PRESS
BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS
LONDON, ENGLAND

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INTRODUCTION

To the student of marine ecology San Francisco Bay presents a series of conditions of unusual interest. A large body of water connected by a narrow passage with the sea, receiving at its upper end the combined discharge of the San Joaquin and Sacramento rivers, and at its lower end the drainage from a broad expanse of tide-marsh, the bay exhibits gentle gradients from salt-water through brackish-water to fresh-water conditions, and from deep-water to shallow-water and salt-marsh habitats, with corresponding changes in temperature, dissolved gases, and associated conditions.

The physical conditions in San Francisco Bay, especially the factors of depth, salinity, temperature, and nature of the bottom, were studied extensively by Sumner, *et al.* (1914), during six observation periods at approximately bimonthly intervals through the course of one year. Supplementary to this is the careful study by Gilbert (1917), dealing particularly with the volume of different portions of the bay, the tidal prism, storage of water on tide-marshes, velocity of current, changes due to silting in the shoals, and the relation of all these to the transportation of suspended matter and its deposition on the bar outside the Golden Gate.

Thus far, however, no intensive study has been made of the chemical conditions of San Francisco Bay waters, of the relation of these to physical conditions, nor of the relations of both to tidal changes, which are of considerable magnitude in San Francisco Bay and must exert a decided influence on the condition of the water—hence indirectly on plant and animal life—through the recurring admixture of bay water with incoming water from the open sea.

It seemed to the present writers that considerable information of both theoretical and practical importance might be gained by a detailed study of the range of physical and chemical conditions of the bay waters at representative localities, based on observations at frequent intervals through a number of tidal cycles. In the summer of 1923 the investigation here reported was accordingly undertaken.

The results are based on analyses of surface and bottom water samples taken at approximately hourly intervals throughout a twenty-

hour (minimum) period, at each of four selected stations on San Francisco Bay. The factors considered are salinity, temperature, turbidity, dissolved oxygen, dissolved hydrogen sulfide, and hydrogen-ion concentration. The program of observations was repeated for a total of three times at each of the four stations. A supplementary study was made at a fifth station where, because of difficulty of access and inadequate facilities, it was not possible to carry out the complete series of observations.

ACKNOWLEDGMENTS

This study represents a phase of the investigations of the San Francisco Bay Marine Piling Committee, being a portion of the hydrographic studies carried on under the auspices of that organization with the primary object of gaining a better knowledge of the environmental conditions influencing the occurrence and distribution of marine wood-boring organisms. With the generous permission of the Committee, the results of this special investigation are here set forth in their broader biological aspects.

The authors are indebted to Professor C. A. Kofoid, of the University of California, for invaluable direction and advice. Thanks are due Professor George F. McEwen, of the Scripps Institution of Oceanography, for helpful information in connection with hydrographic problems; Professor R. S. Holway, of the University of California, for special information on the physiography of San Francisco Bay; Professor W. C. Allee, of the University of Chicago, for suggestions as to apparatus and methods; Professor T. G. Thompson, of the University of Washington, for numerous helpful criticisms and suggestions; and Dr. E. G. Moberg, of the Scripps Institution, for advice regarding methods of hydrogen-ion determination.

The chemicals and apparatus used were provided in part by a grant from the Committee on Marine Piling Investigations of the National Research Council. The major expenses of the investigation were borne by the San Francisco Bay Marine Piling Committee.

DESCRIPTION OF SAN FRANCISCO BAY

San Francisco Bay is an irregularly shaped body of water extending in a direction from north-by-west to south-by-east, for a distance of approximately fifty-two miles. Its maximum width is a little more than ten miles. At a point about two-fifths of the distance from its northern to its southern end, the bay is connected with the open sea by a channel having a minimum width of slightly more than one mile, and a maximum depth of some three hundred feet.

Near its northern end, an arm of the bay extends eastward through the Carquinez Strait, and again widens out to constitute the body of water known as Suisun Bay. The latter, and the strait also, are sometimes omitted in computations of the area and volume of the bay (McAdie, 1913), and the hydrographic studies of Sumner *et al.* (1914), did not include these. There are obvious physiographic reasons for this practice, as Suisun Bay is separated from the main portion of San Francisco Bay by a narrow channel some six miles long. There is, however, no definite faunal or hydrographic break here, but a fairly uniform gradient from salt-water to fresh-water conditions. The present authors have found it both convenient and desirable to regard Suisun Bay as a portion of greater San Francisco Bay, the upper terminus of which may hence be considered as Antioch on the San Joaquin River, and Collinsville on the Sacramento River, in accordance with the practice of Gilbert (1917). The area of San Francisco Bay thus regarded is, at half-tide, about 433 square miles.

This large area of water falls somewhat naturally into three major divisions, which, on the basis of geographical position, may conveniently be termed "upper," "middle," and "lower."

The upper bay, including the areas known specifically as San Pablo Bay, Mare Island Strait, Carquinez Strait, and Suisun Bay, represents the brackish-water environment. This division receives the discharge of the Sacramento and San Joaquin rivers, Napa Creek, Sonoma Creek, Petaluma Creek, and several lesser streams which are nearly or completely dry during several months of the year. The interaction of run-off and tidal movements in this portion of the bay produces a constant fluctuation of hydrographic conditions. The situation is further complicated by seasonal differences in the amount of water discharged by the tributary streams, the run-off ordinarily

being more than five times as great during the first six months of the year as during the last six months (Grunsky, 1921). These conditions have been discussed in detail by Kofoid (1921), and Kofoid and Miller (1922, 1923).

The middle bay may be bounded rather definitely on the north by a line through Point San Pablo and Point San Pedro. Its southern boundary, however, can be only somewhat arbitrarily set. Sumner *et al.* (1914), as a matter of convenience, considered it to be "a line through the Ferry Building and Goat Island Light." On the basis of ecological conditions, however, this line might as well or better be moved several miles farther south, perhaps as far as Hunter's Point. The middle bay as thus bounded is characterized by a higher and more constant salinity, a more equable temperature, and greater depth, than either of the other divisions. The condition of the water here most nearly approaches that of the ocean, with which this segment of the bay is in immediate communication through the Golden Gate. From this point there is naturally a gradient in conditions both north and south.

The lower bay has the greatest area of any of the three divisions, but is characterized by large expanses of relatively shallow water, with broad stretches of tide-marsh adjoining. There are no large streams tributary to this portion of the bay, but several small creeks contribute a sufficient amount of fresh water during the rainy season to appreciably lower the mean salinity, so that it stands intermediate between that of the middle bay and that of the upper division. The entire physico-chemical complex of the lower bay is measurably altered by the effect of meteorological conditions on so large a body of relatively shallow water.

LOCALITIES SELECTED FOR INVESTIGATION

In considering localities for this investigation, first attention was given to the selection of stations that should be representative of the conditions typical of each of the three major divisions of the bay, as outlined above. Consideration had also to be given to such factors as the presence of a wharf, so that water samples could be taken a suitable distance out from shore; the presence of some sort of shelter for the protection of apparatus and chemicals from the weather; and accessibility, so that too much time might not be consumed in getting

from station to station. At best, it was necessary in some instances to transport all equipment fifty miles between a low tide and the following high tide. Thus localities too remote or difficult of access became automatically eliminated.

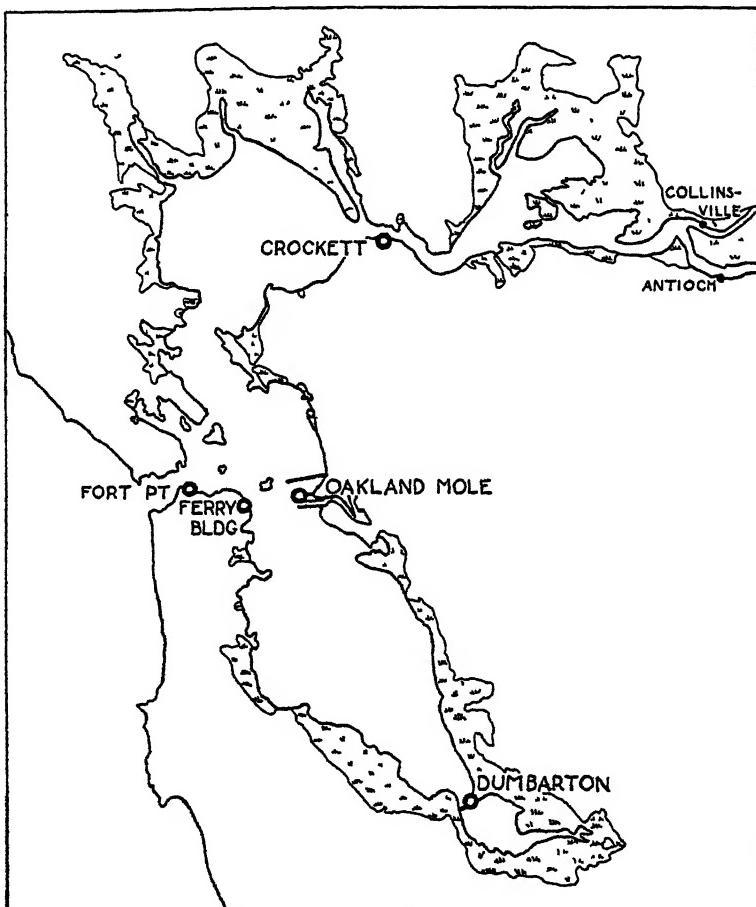


Fig. A. Map of San Francisco Bay, showing localities at which water samples were taken.

As a station representative of conditions in the upper bay, Crockett, at the entrance to Carquinez Strait, was selected. Observations here were carried on from the dock of the California and Hawaiian Sugar Refining Corporation. In the middle bay, two stations, one on each side of the bay, were selected, one at the San Francisco Ferry Building, the other on the end of the Oakland Southern Pacific Mole. In the lower bay the only locality at which it was at all feasible to work

was Dumbarton, where observations were carried on from the drawbridge of the Southern Pacific Company. The difficulties at this location rendered it impossible to carry out the full series of observations planned, but such data as were secured here possess considerable interest.

As a basis for the comparison of these data a full program of observations was carried out at Fort Point, at the southern side of the Golden Gate. It was considered that here the incoming tide would represent most nearly the conditions of ocean water, while the outgoing tide would afford a kind of summary of the total effect of the mixture of ocean water and bay water. This expectation, it will be seen later, was not entirely realized. An additional reason for the selection of this station for an investigation dealing with certain aspects of tidal phenomena was the fact of its being an important tidal station, the times of high, low, and slack waters given in the Coast and Geodetic Survey tables referring specifically to Fort Point.

The five localities thus selected for investigation had all been previously explored on collecting trips by one or more of the authors, so that in a general way the faunal and ecological conditions were known, and the localities were regarded as especially suitable for the purposes of this study.

TIDAL MOVEMENTS IN SAN FRANCISCO BAY

More than one-sixth of the total amount of water present at high tide in San Francisco Bay, according to the computation of Sumner *et al.* (1914), passes out through the Golden Gate during the average ebb tide. The reciprocal of this, namely, that the amount of water present in the bay at low tide becomes mingled with one-fifth of its volume of ocean water during the average flood tide, is obviously a fact of considerable biological significance. In addition to the rhythmical variations in salinity, temperature, and other conditions thus produced within the bay, account must be taken of the mechanical effect of tidal movements in stirring up the water, carrying away débris, dissipating the local effect of sewage contamination, and contributing to increased oxygenation of the water.

Quite as important from the ecological point of view as the average tidal conditions, are the departures from the average, especially the extreme conditions to which sessile or sedentary organisms are

subjected over a period of time. Useful as the arithmetical mean proves itself to be in summing up and comparing different sets of data, the fact must be borne in mind that the mean condition represents an abstract concept that is infrequently realized or even approximated in nature. This is especially true in the case of data involving tidal phenomena, since the tides exhibit pronounced diurnal, monthly, and seasonal inequalities, for which the mean is a wholly inadequate expression.

The principal factors influencing the range of the tides in San Francisco Bay are the coincidence of solar and lunar attractions (producing spring tides), or their opposition (producing neap tides); the distance of the moon from the earth at given times (producing apogee or perigee tides); and the distance of the moon north or south of the plane of the earth's equator (producing tropic tides). The last-named factor is stated (Gilbert, 1917) to be the chief cause of inequality of the two high or two low waters of the same day, giving thus during each lunar day a higher high and a lower high water, and likewise a higher low and a lower low water.

Prevailing winds at times may influence both the height and time of the tides.

The interaction of these more or less independently variable factors determines the range of the tide on any given day. Thus the greatest tides occur when two or more tide-producing factors operate together, and conversely, the tidal range is least when the influence of the several factors involved is so distributed that each has a tendency to counteract the others.

The mean range of the tides at forty-one points in San Francisco, San Pablo, and Suisun bays, according to the tide tables issued by the United States Coast and Geodetic Survey, is 4.83 feet. The average range of all spring tides is approximately 25 per cent greater than this, while the greatest tides have a range about twice as great as the mean.

During the tropic tides of the month, when the diurnal inequality is greatest, the higher high water is followed by the lower low, while the latter is followed by a lower high water and a higher low water before the ensuing higher high tide. Thus it comes about that almost as much water passes out on one great ebb as comes in on two successive floods. This will be made clear by reference to figure B.

It was found by both Sumner *et al.* (1914), and Gilbert (1917), that the average rate of flow of the ebb current is more rapid than

that of the flood. This is due in part to reinforcement of the ebb by run-off, but more particularly to the fact that the sectional area of the channel increases as the water rises, so that at the time of maximum discharge of the flood current, which comes a short time before high water (see fig. B), the sectional area of the channel is considerably greater than at the time of maximum discharge of the ebb current, which comes a short time before low water.

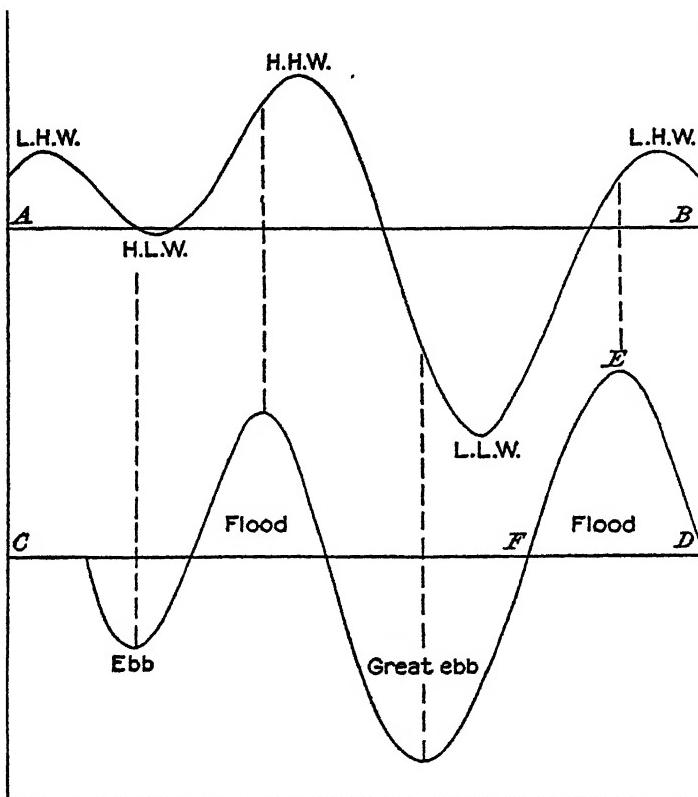


Fig. B. Sequence and relation of tropic tides and currents in San Francisco Bay. The upper curve gives stage of the tide (vertical) in relation to time; the lower gives the corresponding rate of discharge. Flood discharges are laid off upward from CD , ebb discharges downward. *L.H.W.*, lower high water; *H.L.W.*, higher low water; *H.H.W.*, higher high water; *L.L.W.*, lower low water. (From Gilbert, 1917, by permission of the U. S. Coast and Geodetic Survey.)

The velocity in the Golden Gate of the average flood tide was found by Gilbert (1917) to approximate 5.05 feet per second (mean of two tides approaching the average range), while the velocity of the ebb was found to be about 6.4 feet per second. These observations were

made in September, when the effect of run-off would be at a minimum. The average flood current in all portions of the bay was found by Sumner *et al.* (1914), on the basis of a somewhat limited number of observations (142) to be 2.01 feet per second (1.19 nautical miles per hour), while the corresponding ebb velocity was found to average 2.85 feet per second (1.68 nautical miles per hour). The greatest differences between flood and ebb velocities were found in November and December, indicating the reinforcement of the ebb by run-off during the rainy season. No observations were made in January and February, when a still greater discrepancy between flood and ebb velocities would be expected.

It is estimated by Sumner *et al.* (1914), without claim to exactness, that the mean rate of water flow for all phases of the tide over the entire bottom of San Francisco Bay (Suisun Bay not included) is between two-thirds and three-quarters of a nautical mile per hour. The rate of flow of surface and intermediate layers, being somewhat higher than this because of reduced friction, would perhaps approximate one nautical mile (1.15 statute miles) per hour.

From the fact stated above, that about one-sixth of the volume of water in San Francisco Bay at high tide passes out through the Golden Gate on the average ebb, it follows that during the largest tides, which have a range of about twice the mean, between one-fourth and one-third of the amount of water in the bay at high tide passes out to sea on the great ebb, to be replaced by an approximately equal volume of ocean water on the two following floods.

Gilbert (1917) has estimated that the volume of the average tidal flow through the Golden Gate is 52,552,000,000 cubic feet, while the mean volume of the great tropic ebbs is 91,948,000,000 cubic feet. On the other hand, the lesser of the two tidal oscillations of the lunar day effects a comparatively limited interchange of bay and ocean water, which may even at times be almost negligible. The average range of neap tides at Fort Point is 3 feet, or about 25 per cent less than the mean of all tides; but the difference between high and low water is occasionally as small as 0.4 foot.

There is thus to be expected, in addition to the daily rhythmic variations in the condition of the bay water produced by flood and ebb tides, periodic differences in the range of such variations correlated with the major tidal inequalities of the month. It would seem probable that the differences in hydrographic conditions during periods of spring and neap tides, respectively, are of greater magni-

tude than the differences associated with the high and low tides of any lunar day.

It should further be emphasized that the height of the tides as such has no biological significance other than in the irrigation of a larger or smaller area of the intertidal zone. The more important consideration in connection with tidal phenomena is the movement of large amounts of water from ocean to bay or bay to ocean on *flood* or *ebb currents*.

The periods of flood and ebb are not coextensive with the intervals between high and low waters, since, owing to the momentum of a large volume of water in motion, the current continues flowing in a given direction some time after either the crest or trough of the tidal wave has passed. High water thus occurs, not at the end of the flood period, but approximately at the time of greatest velocity of the flood current, although this normal relation is usually modified by local contours. Slack water, or the time of actual reversal of the current, follows high or low tide, after a variable interval depending on the range and velocity of the particular tidal movement involved, as well as on local physiography.

In San Francisco Bay, in general, the interval between a high or low water and the following slack water is from one to two hours. In studying the effect of tidal phenomena on hydrographic conditions, it is important to bear in mind that the maximal concentration of flood water will be reached one or two hours after high tide, while the most concentrated ebb water will be obtained a corresponding period after low tide.

SELECTION OF TIDES FOR THIS STUDY

The tides occurring during the month of July, 1923, seemed particularly favorable for the purposes of this investigation. The two series of spring tides of this month were among the largest tides of the year. At the same time, the tidal range remained fairly constant during five successive days on each of the springs. This allowed observations made at our five stations on as many successive days to be comparable on the same basis so far as tidal range was concerned.

The neap tides of the month were less satisfactory, since their range was greater than the average neap range, and further, the height of high water changed considerably from day to day, introducing a troublesome variable. Thus, while our data on the spring tides

are considered to be properly representative of the greatest effect of the tides on the condition of the bay water, the neap tide observations indicate less uniform conditions than would be expected on the least tides.

Our observations were carried on during three five-day periods, covering two series of spring tides and the intervening neap tide period. Details as to the heights and ranges of these tides are given in the graphs (charts 1-5).

EQUIPMENT AND METHODS

It is considered desirable to devote space here to an adequate discussion of equipment and methods, inasmuch as the authors were at considerable pains to select or devise facilities which should meet the requirements of this type of work, combining the features of ready portability, sufficient accuracy, rapidity of operation, and adaptability to field conditions. The methods and equipment described below proved satisfactory except as noted. For the convenience of other workers, brief but explicit directions for each operation have been included.

The entire equipment could be set up for action, or taken down and packed for transportation, in less than one hour, each piece of apparatus and each reagent having its assigned place in one of four boxes (dimensions about $1 \times 1\frac{1}{2} \times 2\frac{1}{2}$ feet), equipped with rope handles for convenience in carrying. In an emergency all equipment and a supply of chemicals adequate for about five days in the field could be carried a limited distance by two men; but in practice a light automobile was used for transportation.

WATER SAMPLING APPARATUS

The apparatus used for taking the water samples is a modification of that designed by Moore (1910) for quantitative plankton studies. It consists essentially of a brass cylinder of about 2.3 liters' capacity, with a movable top and bottom each fitted with a thick, soft-rubber washer, beveled as shown in figure C (*a*, *a'*). By means of a hole drilled through the top, and a central ring held in position near each end of the cylinder by a metal spider, the top and the cylinder are designed to slide up and down a limited distance along an upright brass rod two feet long, which is screwed firmly into the metal base. The upper end of the rod is bent into a ring for the attachment of a light cable.

The cylinder is suspended from the top section by a short length of brass chain, so that when the apparatus is "set" by hooking the trigger (*c*) over the metal spool, the cylinder is open at both ends, permitting a free flow of water through the apparatus as it is being lowered.

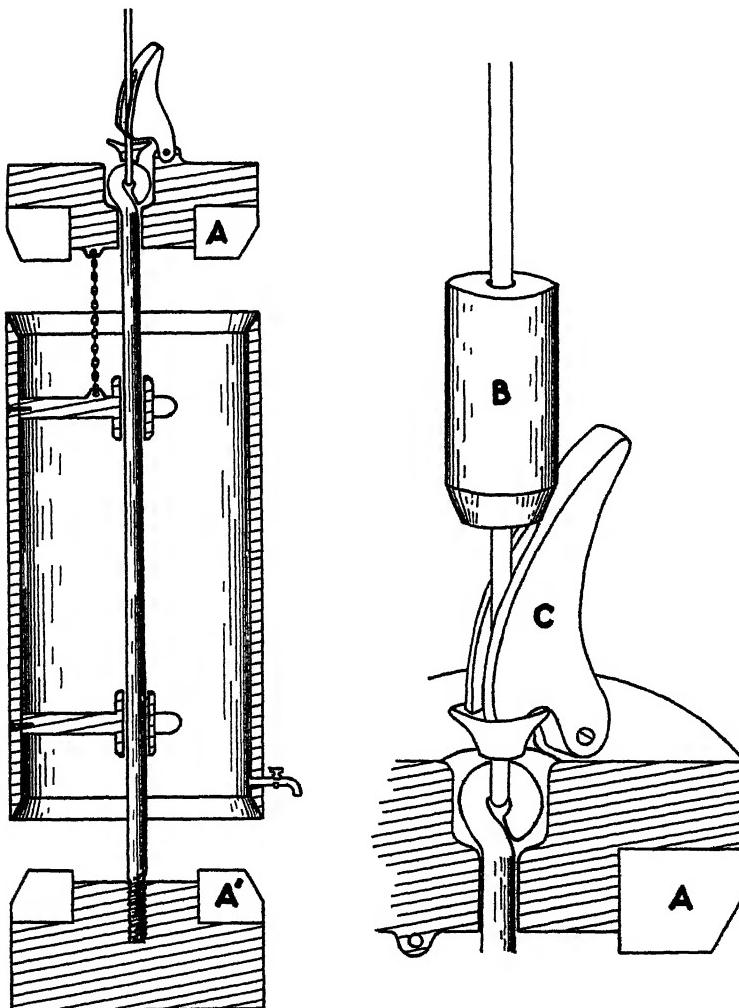


Fig. C. Apparatus used for taking water samples (left), and detail of tripping device (right). For explanation, see text.

The sampler is lowered by a flexible one-quarter inch wire cable to the desired depth, and is closed by a rider (*b*) which slides down the cable, by its impact forcing the trigger off the metal spool.

Thereupon the cylinder falls of its own weight, and is forced tightly over the sloping sides of the base by the added impact of the top section, which is designed to weigh about ten pounds (this is perhaps unnecessarily heavy). The rubber washers minimize the jar when the apparatus closes, and effectively prevent leakage of the sample.

The cylinder was bored near its lower edge and a stopcock attached for withdrawing the water sample. A rubber jacket, improvised from an automobile inner tube, was drawn over the cylinder as a partial insulation against rapid changes in temperature.

The wire cable was paid out from a reel, housed in a box which could be spiked to a pile or the edge of a dock, thus furnishing a windlass. The box housing the cable contained space also for the sampler, so that the whole made a compact portable unit.

This apparatus is simple and entirely satisfactory for use in waters of moderate depth. The tripping device is operated readily by a weight of five ounces falling any distance over three feet. The successful closing of the sampler is indicated by a quite perceptible jerk on the cable.

TEMPERATURE

In taking a water sample, the apparatus was lowered to the desired depth and allowed to remain about two minutes before being closed, in order to bring the cylinder approximately to the temperature of the water. Then the messenger was dropped, the sampler was rapidly drawn up, and the temperature was taken as promptly as possible with a thermometer graduated in tenths of a degree Centigrade.

This method of taking water temperature is open to criticism. But in practice it was found that, with the degree of insulation afforded by the rubber jacket around the sampler, a water sample at 17° C., standing on the dock in the sunshine of a warm July day, showed an increase in temperature of only 0.1° C. in two minutes. It is thought, therefore, that no appreciable change in temperature was produced in bringing bottom samples rapidly through the warmer upper strata of water, or could occur in the brief interval required to take a temperature reading after the sample was brought to the surface.

SALINITY

Salinity samples were subjected to the Mohr method of determination of chlorine—titration with a standard solution of silver nitrate, using potassium chromate as indicator. The silver nitrate solution

was made by dissolving in distilled water 23.9545 gms. of chemically pure silver nitrate, which had first been carefully dried, and diluting the solution with distilled water to a volume of exactly 1000 cc. Using a solution of this strength, and a 5 cc. sample of the sea water, the gms. of chlorine per liter are equal numerically to the cc. of silver nitrate solution required for the titration. The potassium chromate was used as a 5 per cent solution (approximately 50 gms. of K_2CrO_4 dissolved in 1 liter of distilled water). The procedure was as follows:

To 5 cc. of the sample, measured with a carefully calibrated pipette, add about 0.5 cc. of the potassium chromate solution, in a porcelain evaporating dish or an Erlenmeyer flask of convenient size (150 cc.). Dilute with about 25 cc. of distilled water. Add the silver nitrate solution very slowly from a burette, stirring or shaking the sample constantly and vigorously during the addition, until the first permanent reddish tinge appears. The chlorine is then calculated as follows:

$$\text{Gms. Cl per liter (for 5 cc. sample)} = \text{cc. of } AgNO_3 \text{ solution used}$$

$$\text{Parts of Cl per 1000} = \frac{\text{gms. of Cl per liter}}{\text{specific gravity of sample}}$$

The specific gravity can be determined with sufficient accuracy for ordinary purposes by reference to Knudsen's Hydrographic Tables (1901), *assuming* the specific gravity at 17.5° C. to be the same for grams of chlorine per liter as for parts of chlorine per thousand. This assumption, which is obviously incorrect, will occasion a positive error in the second decimal of the result for parts of chlorine per thousand. The result as calculated should therefore be corrected by reference to the following table:

Parts of Cl per 1000.....	5	10	15	20
Correction	-0.01	-0.02	-0.04	-0.06

For greater accuracy and convenience, Thompson (1928) has lately prepared a table for converting grams of chlorine per liter directly to parts of chlorine per thousand, from the empirical formula

$$Cl_w = 0.008 + 0.99980 Cl_v - 0.001228 Cl_v^2$$

where Cl_w represents grams of chlorine per kilogram, and Cl_v represents grams of chlorine per liter at 20° C. Professor Thompson has kindly supplied us with a copy of his manuscript prior to its publication and has consented to this citation.

Emphasis should be placed on the importance of temperature control if accurate results are to be obtained by the volumetric method. The use of Knudsen's tables in the calculation described above assumes a temperature of 17.5° C. Thompson's table is based on a temperature of 20° C. It is usually more convenient to bring the water to one of these standard temperatures before measuring off the sample than to apply a correction for temperature subsequently.

If the sample for analysis be weighed instead of measured (which, however, is not feasible under field conditions), all data are on the weight basis, and the calculation becomes simply:

Parts of Cl per 1000 (for a 5 gm. sample) = cc. of AgNO₃ solution used.

By reference to Knudsen's Hydrographic Tables (1901), the parts of chlorine per 1000 can be read directly into total salinity, or the salinity can be calculated from the following formula:

$$S = 1.8050 \text{ Cl} + 0.030.$$

The silver nitrate solution should be checked against pure dry sodium chloride before use if it has been made up for more than a few days. It is usually more convenient to make up only enough for immediate needs, and to make up fresh solution when necessary. In any case, however, where extremely accurate results are desired, the silver nitrate should be checked before use. 0.2061 gms. of NaCl dissolved in a few cc. of distilled water should require for titration exactly 25.00 cc. of a silver nitrate solution made up as above described.

The accuracy of the Mohr method has been called in question by Sumner *et al.* (1914), Michael and McEwen (1915), and others. On the other hand, the method is accepted as standard in the latest texts on quantitative chemistry, and is in general use in analytical laboratories. Its use for oceanographic purposes has recently been defended by Smith and Thompson (1927), who obtained close checks on analyzing samples of the same sea water by the gravimetric method, Mohr's method, and Volhard's method.

It has been claimed (Fales, 1925, p. 199) that, for satisfactory results with the Mohr method, the Sörensen value of the sample should not be below pH 4.0, nor much above pH 7.0, although a recent paper by Van Urk (1925) indicates that the upper limit may safely be considerably extended. In any case, as the Sörensen value of sea water is rarely much above pH 8.0, the above recommended dilution with distilled water, which usually contains a considerable amount of carbon dioxide, is probably sufficient to bring the sample safely within the required range.

The Mohr method is, of course, simply a method for determining chlorine in solution. Its validity as an index of total salts depends on the assumption of the uniformity of the composition of the ocean waters, in different localities and at various dilutions. That this assumption, with the exception of certain special cases, is justified, is shown in a full review of the data on analyses of sea water by Clarke (1924, pp. 125 ff.), and by the recent analyses by Thompson *et al.* (1927), of waters of the North Pacific.

DISSOLVED OXYGEN

The sample for oxygen determination was drawn from the bottom of the sampling apparatus by means of a flexible rubber tube attached to the stopcock at the bottom of the cylinder. The free end of the tube was inserted in a bottle of the type known to the trade as "citrate of magnesia bottles" (capacity about 250 cc.), which was gently filled and allowed to run over a sufficient length of time to flush the bottle out twice. The necessary reagents (see below) were then immediately added, and the bottle was stoppered. Care was taken to insert the rubber tube to the bottom of the bottle, to avoid splashing as the water ran in, and to avoid inclusion of air bubbles in stoppering the bottle. With these precautions, the possibility of contamination of the sample with atmospheric oxygen is exceedingly remote.

As a matter of fact, the authors consider that the precautions usually recommended in taking water samples for determination of dissolved oxygen are more elaborate than is strictly necessary. The rate of diffusion of atmospheric oxygen into water is exceedingly slow (cf. Birge and Juday, 1911, p. 60), and inasmuch as the dissolved oxygen content of most littoral waters approximates saturation, it would seem that the likelihood of contamination by atmospheric oxygen has been somewhat overemphasized. Even in waters that are supersaturated, or that contain only minute amounts of dissolved oxygen, ordinary precautions to avoid splashing or shaking the sample, to add the chemicals promptly, and to avoid using the stratum of water that has been immediately in contact with the air, are sufficient to secure a representative sample.

Dissolved oxygen was determined by the Winkler method, for which the following reagents are necessary:

1. Manganese sulfate solution. Approximately 450 gms. of $MnSO_4 \cdot 4H_2O$ (or 400 gms. of $MnCl_2 \cdot 4H_2O$) in a total volume of 1 liter. (The sulfate is preferable to the chloride, because of the difficulty of obtaining the latter free from traces of iron.)

2. Alkaline iodide solution. Approximately 420 gms. of NaOH (or 590 gms. of KOH) and 100 gms. of KI in a total volume of 1 liter. The alkali used in making up this solution must be nitrate free; otherwise the end point will not be sharp in the final titration, and the results obtained will be too high.

3. Concentrated hydrochloric acid.

4. N/100 sodium thiosulfate solution. 2.482 gms. of chemically pure $Na_2S_2O_3 \cdot 5H_2O$ in a total volume of 1 liter. Should be standard-

ized frequently (at least twice a week under average conditions) against N₁/100 sodium bichromate solution.

5. Starch solution. Approximately a 1 per cent solution, containing a few drops of chloroform as a preservative.

Before the sample is stoppered, add with a long-stemmed pipette extending nearly to the bottom of the bottle, 2 cc. of solution No. 1. In the same way add 2 cc. of solution No. 2. Stopper the bottle and shake thoroughly. After the precipitate has settled, add 2 cc. of solution No. 3, restopper and shake until the precipitate has dissolved. Transfer the sample to a 500 cc. Erlenmeyer flask and titrate the liberated iodine with solution No. 4, added from a burette, until the iodine color has diminished to a faint yellow. Add 1 or 2 cc. of the starch solution as an indicator, and continue the titration till the sample is colorless.

The *apparent* amount of oxygen can now be calculated from the volume of the thiosulfate solution used. To obtain the true value, however, we must add a correction equal to the oxygen equivalent of the hydrogen sulfide present, which must be separately determined (see below). The dissolved oxygen therefore is calculated as follows:

$$\text{Gms. of O}_2 = \frac{\text{cc. N } 100 \text{ sod. thiosulf.} \times 0.08}{\text{per liter vol. of sample bottle in cc.} - \frac{1}{4}^*} + 0.47 \times \text{gms. of H}_2\text{S per liter.}$$

* Correction for water displaced by reagents added.

It is customary to express dissolved oxygen either as parts per million (=mg. per liter), or as cc. per liter (=gms. per liter \times 700) at standard temperature and pressure.

For waters considerably polluted, it is necessary to use the Rideal-Stewart modification of the Winkler method (see Am. Publ. Health Assoc., 1925, p. 59). When this method is used, the correction for hydrogen sulfide in the above calculation is eliminated.

DISSOLVED HYDROGEN SULFIDE

Dissolved hydrogen sulfide was determined by adding to a sample of the water an excess of N₁/100 iodine solution, and titrating the excess iodine with N₁/100 sodium thiosulfate solution. This method does not give an exact measure of hydrogen sulfide, since organic compounds may react with the iodine, give a somewhat higher value than that of the dissolved hydrogen sulfide alone. On the other hand, the presence of appreciable amounts of nitrates in the sea water may liberate iodine from the potassium iodide, causing a small error in the opposite direction. However, the method does give a fairly close measure of the reducing materials present in the water, and hence is desirable as a reciprocal of the Winkler method of oxygen determination, as explained above. Since the amount of these materials is usually small, a fairly large sample should be used. The

results obtained by this method are considered to afford a fairly satisfactory index of the degree of pollution of the water, by sewage or other organic wastes.

The following reagents are required:

1. Standard N/100 iodine solution. Dissolve approximately 25 gms. of KI and 1.27 gms. of resublimed iodine in a total volume of 1 liter. Standardize this solution against solution No. 2.
2. Standard N/100 sodium thiosulfate solution. This is the same as solution No. 4 used in determining dissolved oxygen.
3. Starch solution. Approximately a 1 per cent solution (solution No. 5 above).

To a measured sample of the water (250 cc.) add from a burette enough of solution No. 1 to impart a distinct yellow color. Note volume used. Add 2 cc. of the starch solution, and titrate to colorlessness with solution No. 2. The amount of hydrogen sulfide is calculated as follows:

$$\text{Gms. of H}_2\text{S} = \frac{(\text{cc. N/100 iodine} - \text{cc. N/100 sod. thiosulf.}) \times 0.17}{\text{volume of sample in cc.}}$$

= [for a 250 cc. sample]:

$$(\text{cc. N/100 iodine} - \text{cc. N/100 sod. thiosulf.}) \times 0.00068$$

It is customary to express dissolved hydrogen sulfide either as parts per million (= mg. per liter), or as cc. per liter at standard temperature and pressure (= gms. per liter $\times 657$).

Hydrogen sulfide is rather unstable, especially in the presence of dissolved oxygen. The iodine solution should, therefore, be added as soon as possible after taking the sample.

HYDROGEN-ION CONCENTRATION

The hydrogen-ion concentration of the water was determined colorimetrically by means of the double-wedge comparator, a method described by Bjerrum (1914), and improved by Barnett and Barnett (1920). The apparatus consists essentially of a narrow glass trough divided by a diagonal glass partition into two wedge-shaped compartments, into which are introduced solutions exhibiting, respectively, the two extremes of the range of color of the indicator which it is desired to use. By looking at the trough from the side, the observer sees the combined color effect of the two isolated solutions, the trough presenting from one end to the other a gradual change in color through the complete range of the indicator used.

A sample to be tested is placed in a square glass cup of the same fluid diameter as the trough, a measured quantity of indicator is added

to bring the concentration of indicator of the sample to that of the standard, and the cup is moved along the top of the trough to a point at which a color match is obtained. The Sörensen value (pH) of the sample is calculated from a scale on the side of the comparator, the value of the mid-point on this scale being the dissociation constant of the indicator used.

This method has the important advantage of eliminating the use of buffer solutions, which are troublesome to prepare, and the stability of which can be guaranteed for only a limited period of time. It has also the advantage of greater accuracy than can be obtained by the use of ordinary standard tubes. An accuracy of .02 Sörensen unit has been claimed for the comparator by Barnett and Barnett (1920), although the present authors consider that, under field conditions, the method is possibly not more accurate than .05 Sörensen unit (i.e., $\pm .025$). With tube standards, an accuracy of this order can only be approximated by interpolation.

The selection of an indicator is determined by the range of Sörensen values likely to be encountered, which can be quickly approximated by the use of one of the so-called "universal indicators" that are on the market. The most useful single indicator for sea water is cresol red (orthocresolsulfonphthalein), which has a range of about pH 7.2 to 8.8. All Sörensen values of San Francisco Bay waters found by the authors have been within the useful range of this indicator. Brom-thymol blue (range pH 6.0 to 7.6) and thymol blue (range pH 8.2 to 9.8) were constantly carried, but were never called into use.

In making up the standard acid and alkaline colors for use in the two compartments of the comparator, a few cc. respectively of 10 per cent acetic acid and of a 10 per cent solution of sodium carbonate were found more satisfactory than smaller quantities of the stronger acids and bases. The standards are made up each in a measured quantity of distilled water (e.g., 50 cc.), to which enough indicator is added to impart a very definite color. The number of drops of indicator used should be counted, as it is important to have the same concentration of indicator in each wedge of the comparator, and in the sample to be tested. The acetic acid and sodium carbonate solutions will produce the full acid and alkaline colors of the indicator used, and have sufficient buffer action to prevent any change in color through absorption of carbon dioxide from the atmosphere, or by the interchange of small amounts of the solutions in the two compartments by accident, or through capillarity.

Barnett and Barnett (1920) have recommended thin, high-grade glass in the manufacture of a comparator; but we have used strips of plate glass, which are less likely to be broken, and present wider edges which are more easily and firmly cemented together. The slight greenish cast of the plate glass is not considered to introduce any error in judging the colors of the solutions, as the same glass was used for the trough as for the cup containing the sample.

The strips of glass were cemented together with china cement (Canada balsam or shellac are quite as satisfactory, but require longer to dry) and all seams were coated with melted paraffin. Even with this last precaution, the glass strips showed a tendency to soak apart after a time, and, though they could be readily cemented together again, it was necessary to carry a duplicate comparator to insure an uninterrupted record of Sörensen values.

As work had to be carried on both by day and by night, it was thought necessary, in order to secure uniform results, to use artificial light for all colorimetric work, the source of light being a "daylight bulb," the rays from which were diffused by a square of frosted plate glass. At Dumbarton, where electric light was not available, determinations of Sörensen values were made by ordinary daylight, and omitted at night.

All comparator readings have been corrected for salt error according to the following table (from Ramage and Miller, 1925):

TABLE 1
THE SALT ERROR OF CRESOL RED AT SALINITIES FROM 5 TO 35 PARTS PER 1000
OF SEA SALTS

Salinity	pH Correction	Salinity	pH Correction
5	-.11	21	-.24
6	-.13	22	-.24
7	-.14	23	-.25
8	-.15	24	-.25
9	-.16	25	-.25
10	-.17	26	-.25
11	-.18	27	-.26
12	-.19	28	-.26
13	-.20	29	-.26
14	-.21	30	-.26
15	-.21	31	-.26
16	-.22	32	-.27
17	-.22	33	-.27
18	-.23	34	-.27
19	-.23	35	-.27
20	-.24		

TURBIDITY

Turbidity was determined by comparison of a sample of the water with a series of standards made up by suspending in distilled water known amounts of finely divided infusorial earth. The standards were kept in sealed vials 2 cm. in diameter. Comparison of the sample with the standards was made by transmitted light. The water sample for determination of turbidity was taken from the sampler promptly, before any settlement could occur, and the standards were, of course, thoroughly shaken each time before use. The standards were prepared as follows:

A quantity of infusorial earth is passed through a 200 mesh screen, then shaken up with distilled water and allowed to settle for one hour. The water, together with the material which has not settled, is discarded. This is repeated several times, or until all of the very fine material has been discarded and a clear supernatant liquid is obtained after one hour's settling. The resulting material is dried at a low temperature and carefully reground just enough to pass the 200 mesh screen. The standards are then prepared by adding weighed amounts of this material to distilled water (table 2). The turbidity is recorded as parts of silica per million, in accordance with the usage adopted by the United States Geological Survey (1902; or see Am. Publ. Health Assoc., 1925).

TABLE 2
PREPARATION OF STANDARDS FOR DETERMINATION OF TURBIDITY

Distilled water cc	Infusorial earth gms.	Index of turbidity
100	0.002	20
100	0.005	50
100	0.010	100
100	0.020	200
100	0.040	400
100	0.080	800
100	0.100	1000

The turbidity of tubes so prepared slowly changes with time on account of flocculation, especially if the fines have not been carefully removed as described above. The flocculation can, however, be largely prevented, so that the tubes will remain constant for months, if a very small amount of ammonia is added to the water used.

This method has the advantages that the standards are readily prepared, are convenient for field use, and can be duplicated at any time. The method can also be elaborated to any extent desired by using a larger number of standards. For turbidities below 20, tubes of greater fluid diameter must be used.

PROCEDURE

Each station in our series, with the exception of Dumbarton, as noted above, was visited for a period of from twenty to twenty-three hours at three different times during the month of July. This length of time permitted a record of observations covering two high and two low waters, with an overlap of one or two hours at the beginning and end of the run. In general, observations at each station were begun an hour before a high water, carried on through two high and two low waters, and continued to include a slack water following.

Water samples were taken at approximately hourly intervals, the exact time being determined by dividing the interval between successive tidal peaks into equal periods corresponding to the nearest number of solar hours. Two men working steadily were able to take a surface and bottom sample and make the determinations of temperature, turbidity, salinity, dissolved gases, and hydrogen-ion concentration in slightly less than one hour. With three men available, a schedule of sixteen hours out of twenty-four for each allowed the work to be carried on uninterruptedly for five days at a time. Then, after an interval of one or two days, the schedule was repeated.

Surface water samples were taken about one foot below the actual surface of the water, and bottom samples one foot above the bottom, the sampler being lowered until it touched the bottom, and then drawn up a short distance to avoid including any mud or bottom débris in the sample. A stratum of water 18 inches in depth was enclosed by the sampler.

On taking a sample, the sampling apparatus was set on a box to facilitate drawing water from the stopcock at the bottom, the cover was raised only as much as was necessary to insert a thermometer, and the temperature was promptly taken. This occupied about one minute. Then a few cc. of water were drawn for determination of turbidity, after which the sample for oxygen determination was taken off, and the reagents immediately added. Finally about a half-liter was drawn off into a flask, to be divided into samples for determination of hydrogen sulfide, salinity, and hydrogen-ion concentration.

The time required for these operations, coupled with the fact that the sampler was allowed to remain in the water two minutes to bring the metal to approximately the temperature of the sample, occasioned

an interval of about five minutes between taking surface and bottom samples. The surface samples were taken at the times stated in the results, and the bottom samples about five minutes later.

RESULTS AND DISCUSSION

To avoid the introduction of a burdensome amount of tabular material, the detailed results from each station are presented in graphic form in charts 1 to 5, to which constant reference should be made throughout the following discussion. The times of high and low waters indicated on the graphs are the times predicted in the tide tables for the station concerned. The figures for height of tides are likewise the predicted ones, but apply specifically to Fort Point and the Presidio (the height of the tide is the same at both stations), and would be somewhat different for each of the other stations. Inasmuch as the range of the tide at Fort Point indicates the total volume of the water entering and leaving the bay, it is not considered important to take account of the local variations in the height of the water at the other stations.

We are informed by Mr. R. L. Faris, Acting Director of the United States Coast and Geodetic Survey, that the actual times of high and low waters, as recorded by the tide gauge at the Presidio, differed in some cases from the predicted times by from 10 to 31 minutes, 97 per cent of the observed times agreeing with the predicted times within 20 minutes. As our observations were made at approximately hourly intervals only, a difference of one-half hour or less in the time of occurrence of a tidal peak is of little importance. The actual heights of high and low tides also differed from the predicted heights. As registered on the tide gauge they were from 0.1 foot to 0.8 foot lower than predicted. The discrepancy was greatest in the case of the lower low waters, so that the daily range between higher high and lower low water was 0.1 foot to 0.4 foot greater than predicted. The actual ranges are given in the explanation of the charts.

The predicted times of slack water, from the current tables issued by the Coast and Geodetic Survey, together with the durations of the corresponding flood and ebb currents, are indicated on the charts. Mr. Faris states that no record of the actual times of slack water during this period is available.

AVERAGE CONDITIONS AT EACH LOCALITY

For purposes of comparison in the discussion which follows it is convenient to refer frequently to average conditions. The arithmetical means of the results for each station, for each run and for all runs, are accordingly presented in table 3.

CONDITIONS AT EACH STATION CONSIDERED IN RELATION TO THE TIDES

In the following pages an attempt has been made to correlate the maximum and minimum values for each condition studied with stages of the tide, or rather, with flood and ebb currents. For reasons explained on page 211, it is to be expected that the maximum effect of a flood current will be realized during the period from the middle of flood to one or two hours after the end of flood, since the early ebb water is simply late flood water beginning to move in the opposite direction. Similarly the maximal effect of the ebb current may be realized at any time from the middle of ebb to one or two hours after low water slack. Accordingly, in the following pages, a maximum or minimum value occurring during the *middle flood*, *late flood*, or *early ebb* is attributed to the effect of flood conditions, while a maximum or minimum value recorded during *middle ebb*, *late ebb*, or *early flood* is regarded as the effect of ebb conditions.

It is desirable to deal first with conditions at the upper and lower ends of the bay, because of their joint influence on conditions in the middle bay, and also because they exhibit the most definite relations to tidal changes. The five stations at which observations were carried on will accordingly be discussed in the following order: Crockett, Dumbarton, the Oakland Mole, the San Francisco Ferry Building, and Fort Point—i.e., in the reverse order of their proximity to the Golden Gate.

CROCKETT

The water of a bay or estuary represents a mixture in varying proportions of ocean water and shore water derived from such sources as run-off, sewage discharge, and seepage. The most important single factor to be considered in a study of such waters is, accordingly, salinity, which varies in a definite way with the dilution of the ocean water, and is uninfluenced by various conditions that may alter the less stable factors of dissolved gases and hydrogen-ion concentration.

TABLE 3
AVERAGES OF DATA FOR EACH RUN AT EACH STATION, AND FOR ALL RUNS

	Crockett	Dumbarton	Oakland Mole	Ferry Building	Fort Point
Salinity parts per 1000:					
First run—					
Surface.....	13.74		26.35	27.79	29.81
Bottom.....	15.30		26.65	27.91	29.77
Average.....	14.52		26.50	27.85	29.79
Second run—					
Surface.....	14.58	27.78	26.78	29.07	30.89
Bottom.....	16.88	27.85	27.06	29.26	31.17
Average.....	15.73	27.82	26.92	29.17	31.03
Third run—					
Surface.....	17.75	28.36	27.92	29.53	30.64
Bottom.....	19.24	28.59	28.12	29.66	30.82
Average.....	18.50	28.48	28.02	29.60	30.73
All runs—					
Surface.....	15.36	28.07	27.01	28.80	30.45
Bottom.....	17.14	28.22	27.28	28.94	30.59
Average.....	16.25	28.15	27.15	28.87	30.52
Temperature °C.:					
First run—					
Surface.....	18.5		17.2	14.9	13.6
Bottom.....	18.4		16.7	14.6	13.2
Average.....	18.5		17.0	14.8	13.4
Second run—					
Surface.....	19.4	20.1	18.0	15.3	14.4
Bottom.....	19.1	20.1	17.4	15.2	14.4
Average.....	19.3	20.1	17.7	15.3	14.4
Third run—					
Surface.....	19.6	21.1	18.9	15.7	14.7
Bottom.....	19.4	21.1	18.5	15.6	14.5
Average.....	19.5	21.1	18.7	15.7	14.6
All runs—					
Surface.....	19.2	20.6	18.0	15.3	14.2
Bottom.....	19.0	20.6	17.5	15.1	14.0
Average.....	19.1	20.6	17.8	15.2	14.1
Dissolved O ₂ cc. per liter:					
First run—					
Surface.....	5.47		5.34	5.33	5.19
Bottom.....	5.36		5.39	5.24	5.10
Average.....	5.42		5.37	5.29	5.15
Second run—					
Surface.....	5.30	4.84	5.53	5.07	5.27
Bottom.....	5.16	4.79	5.36	5.08	5.43
Average.....	5.23	4.82	5.45	5.08	5.35

TABLE 3—(Continued)

	Crockett	Dumbarton	Oakland Mole	Ferry Building	Fort Point
Dissolved O ₂ cc. per liter— <i>Cont.</i>					
Third run—					
Surface.....	5.63	4.52	6.14	5.37	5.55
Bottom.....	5.50	4.31	5.86	5.32	5.43
Average.....	5.57	4.48	6.00	5.35	5.49
All runs—					
Surface.....	5.47	4.68	5.67	5.26	5.34
Bottom.....	5.34	4.55	5.54	5.21	5.32
Average.....	5.41	4.62	5.61	5.24	5.33
Dissolved H ₂ S cc. per liter:					
First run—					
Surface.....	0.22		0.22	0.13	0.07
Bottom.....	0.25		0.19	0.13	0.07
Average.....	0.24		0.21	0.13	0.07
Second run—					
Surface.....	0.15	0.14	0.15	0.17	0.10
Bottom.....	0.17	0.14	0.15	0.17	0.10
Average.....	0.16	0.14	0.15	0.17	0.10
Third run—					
Surface.....	0.09	0.09	0.10	0.09	0.06
Bottom.....	0.09	0.09	0.10	0.09	0.06
Average.....	0.09	0.09	0.10	0.09	0.06
All runs—					
Surface.....	0.15	0.12	0.16	0.13	0.08
Bottom.....	0.17	0.12	0.15	0.13	0.08
Average.....	0.16	0.12	0.16	0.13	0.08
Hydrogen-ion concentration (pH):					
First run—					
Surface.....	7.69		7.91	7.83	7.95
Bottom.....	7.65		7.88	7.83	7.95
Average.....	7.67		7.90	7.83	7.95
Second run—					
Surface.....	7.59	7.83	7.93	7.86	7.92
Bottom.....	7.59	7.82	7.92	7.86	7.95
Average.....	7.59	7.83	7.93	7.86	7.94
Third run—					
Surface.....	7.75		7.98	7.98	7.94
Bottom.....	7.77		7.95	7.94	7.98
Average.....	7.76		7.97	7.93	7.96
All runs—					
Surface.....	7.68	7.83	7.94	7.87	7.94
Bottom.....	7.67	7.82	7.92	7.88	7.96
Average.....	7.68	7.83	7.93	7.88	7.95

This is well illustrated by the data from Crockett, where, during three observation periods, the salinity rose and fell almost as regularly as the tides, being highest about the end of flood, when the concentration of ocean water is greatest, and lowest about the end of ebb, when the river water preponderates. There is further a marked difference in the effect on salinity of the two tidal oscillations of each day, the highest salinities occurring soon after the highest tide, and the lowest salinities following the great ebb.

Since the bay water has a higher specific gravity than the river water, the mixture of the two is not immediately complete, but the river water tends to flow out on top of the bay water, resulting in a noteworthy difference between surface and bottom conditions. Salinities at the bottom (in 31 feet of water on a zero tide) are generally higher than those at the surface by from 1 to 5 (average for all runs 1.78) parts per 1000; but once during each run, about the middle of a large ebb, the surface and bottom salinities nearly or entirely coincide for a period of one or two hours. It will be observed in the graphs that there occurs at the same time a practical coincidence of surface and bottom values for temperature, dissolved gases, and hydrogen-ion concentration. This indicates that the maximum velocity of the ebb current is sufficient to effect a thorough mingling of surface and bottom waters. But as soon as the velocity of the current has abated on the late ebb, a stratification of the water on the basis of differential specific gravity is reestablished.

A noteworthy feature of the salinity results is the progressive increase in salinity from the earlier to the latter part of the month, the averages in parts per 1000 for the three runs being respectively (surface and bottom averaged together), 14.52, 15.73, and 18.50 (see table 3). This is due to the gradual decrease in river discharge incident upon decreased run-off and the diversion of water for irrigation purposes. This condition is reflected, although in less degree, in increased salinities throughout the bay, as will be noted from a consideration of the salinity results from each of the other stations in our series.

The temperature of the water at Crockett bears a reverse relation to the salinity, the river water at this season of the year being a few degrees warmer than the bay water. The bottom temperatures are consistently highest on the ebb tide and lowest on the flood, with a single exception near the beginning of the third run; but the surface temperatures are somewhat erratic, owing apparently to the immediate

effect of air temperatures on the upper stratum of water. Thus the highest surface temperature on each run occurs on a late flood, which is contrary to expectation; but in each of these instances the temperature in question occurs during the afternoon or early evening of a warm day. Conversely, the lowest surface temperature recorded during the second run occurred at the end of ebb; but this was at 3:41 A.M., when the temperature of the air (16.3° C.) was nearly three degrees lower than that of the water (19.0° C.). Thus it appears that variations in the temperature of the surface water are most immediately related to atmospheric temperatures, but variations in the temperature of the bottom water are definitely associated with tidal changes.

The mean difference between surface and bottom temperatures is very small, surface temperatures averaging only 0.2° C. higher than bottom temperatures for all runs.

It will be noted that there is a progressive increase in both surface and bottom temperatures from the first to the last runs (see table 3 and chart 1), indicating a gradual response of the water to the atmospheric temperatures of summer. The maxima increase more markedly than the minima, showing that the river water is being warmed more rapidly than the bay water.

The amount of dissolved oxygen in the water at Crockett is usually highest about the end of ebb and lowest about the end of flood, indicating that the water flowing in from the rivers has more oxygen in solution than the water of San Pablo Bay in general. This relation holds for the first and third runs, but during the second run is reversed in three out of a possible four cases, constituting an anomaly which can perhaps be partly explained by the influence of solar radiation on photosynthesis, the highest surface value for oxygen occurring in the early evening, and the lowest value at 4:43 A.M. This will be fully discussed on a subsequent page. The average amount of dissolved oxygen present during the first run is greater than during the second run, but less than during the third run.

The dissolved oxygen content of the surface water is greater than that of the bottom water by an average amount of 0.13 cc. per liter, but occasionally coincides with or drops below that of the bottom water.

The dissolved hydrogen sulfide in the water at Crockett during the first run is lowest soon after high tide, and highest soon after low tide, indicating a greater hydrogen sulfide content of the river water than

of the bay water. During the second and third runs the amount of dissolved hydrogen sulfide progressively decreases, and the correlation is less obvious; but in each case there is an upward trend of the curve near the end of the run, on an ebb tide, which accords with the explanation given.

The results for hydrogen sulfide at Crockett on the first run are the highest found at any of our stations (see table 3). But, even so, the amount of this gas in solution in the water is nearly negligible, being less than 5 per cent of the volume of dissolved oxygen present. On the last run the amount of hydrogen sulfide in the water has decreased to less than 2 per cent of the amount of dissolved oxygen present.

The dissolved hydrogen sulfide content of the bottom water at Crockett is higher than that of the surface water by an average amount of 0.02 cc. per liter for all runs. This does not entirely accord with the fact that the bottom water represents the greatest concentration of bay water, which is considered to have a lower hydrogen sulfide content than the river water; but it may be explained by assuming that the bottom river water has a decidedly higher amount of dissolved hydrogen sulfide than the surface river water, which is obviously the case on every late ebb.

With the exception of salinity, the hydrogen-ion concentration at Crockett varies more regularly with the tidal changes than any other condition, the pH values being consistently highest about the end of flood, and lowest about the end of ebb. This is due, of course, to the higher alkalinity of the salt water. The hydrogen-ion concentration here apparently has no immediate relation to any other condition; but in a general way the results for this factor parallel those for oxygen, being higher on the first run than on the second run, and higher on the last run than on either of the others.

The differences between surface and bottom results for hydrogen-ion concentration are erratic, and the minute difference in the final averages (surface, pH 7.68; bottom, pH 7.67) is not regarded as significant.

DUMBARTON

At Dumbarton, it is interesting to note, the relation of salinity to the tides is precisely the opposite of that at Crockett, the salinity of the water decreasing on the flood tide, and increasing on the ebb. During each of the two runs at this station the lowest salinities occur

within one or two hours of a higher high water, and the highest salinities at or near the times of low water slack. This can mean only that the salinity in the broad, shallow expanse of water south of Dumbarton is measurably increased in summer by evaporation. This finding bears out a suggestion of Sumner *et al.* (1914, p. 85), which has been questioned by one of us (Miller, 1922, p. 304).

The range of salinities at Dumbarton is less than at any other of our stations, the greatest range for one run being 2.33 parts per 1000, and the total (bottom) range for both runs at this locality being only 2.63 parts per 1000. As the range of salinities during each observation period must be due to the salinity differential of waters to the north and south of Dumbarton, we conclude that the effect of evaporation on the body of water to the southward is sufficient to increase its salinity by from one to two parts per 1000.

Notwithstanding the considerable depth at this station, 40 feet at zero tide, the differences between surface and bottom salinities are very slight, the bottom salinities for two runs averaging only 0.15 parts per 1000 higher than the surface salinities. This is explained by the fact that Dumbarton is on a narrow channel connecting two larger bodies of water. The tidal currents flow through this channel with extraordinary rapidity, churning up the water and resulting in a fairly homogeneous condition from surface to bottom.

The average salinities of the last run at Dumbarton are very slightly higher than those of the first run.

The temperature of the water at Dumbarton is higher than at any other locality in our series (see table 3). The maximum temperatures show little correlation with stages of the tide, there being probably no marked difference in the temperature of the water in the area south of Dumbarton and that immediately north. The minimum temperatures occur generally about the end of flood, owing to the influence of the cooler water flowing down from the middle portion of the bay.

The differences between surface and bottom temperatures are small, and follow no regular order. The highest surface temperatures are higher than the bottom temperatures, and the lowest surface temperatures lower, reflecting the influence of atmospheric temperatures on the surface water. The averages of surface and bottom temperatures are identical, 20.1° C. on the second run, and 21.1° C. on the third run.

The dissolved oxygen is regularly highest on a late flood tide, although, rather anomalously, on the first run it drops rapidly about the time of each high water slack. On both runs the amount of dis-

solved oxygen drops precipitously on the great ebb, indicating a very low oxygen content of the water from the tide marshes in the southern end of the bay.

The oxygen content of the bottom water is slightly but rather regularly lower than that of the surface water, the average difference for two runs being 0.13 cc. per liter.

The results for oxygen at Dumbarton are lower than those at any other station in our series. Also, the averages for the last run are lower than those for the earlier run, which is the reverse of the condition found elsewhere throughout the bay. These facts may be explained in part by the temperature of the water at Dumbarton, higher temperatures having a tendency to decrease the amount of dissolved gases in solution (see p. 255). The average temperature of the water at this locality is higher than elsewhere in the bay, and the average increase between the second and third runs is greater than at any other station except the Oakland Mole. In general, however, the influence of temperature on the solubility coefficient of oxygen appears to be more than offset by its opposite effect on the rate of photosynthesis (see p. 252). It seems reasonable therefore to assume the presence of an unusually large proportion of zooplankton or other oxygen-consuming organisms in this portion of the bay.

That the lack of oxygen is not due to pollution, or to the presence of large amounts of reducing substances in the water, is indicated by the results for dissolved hydrogen sulfide, which are lower at Dumbarton than at any other station except Fort Point. The data are meager, and as they stand they show no constant differences between surface and bottom conditions, and no correlation with tidal changes. The hydrogen sulfide content of the water is, however, distinctly lower on the last run than on the one preceding.

The data for hydrogen-ion concentration at Dumbarton are very fragmentary, for reasons previously explained (p. 221), but even so they are of considerable interest. On the first run the Sörenson value of the surface water is highest at the time of higher high water, and lowest at the time of lower low water; that of the bottom water is highest at the time of a lower high water, but is unexpectedly lowest just after a higher high water.

On the second run, the few determinations of Sörenson value that were made had to be discarded, as the standards were later found to be inaccurate. They indicate, however, that the hydrogen-ion con-

centration was lower at the end of this run, on a great ebb, than at the beginning of the run, by 0.2 Sörenson unit.

The Sörenson values appear to be most closely related to the results for dissolved oxygen, and, like the latter, are lower at Dumbarton than elsewhere in the bay (Crockett only, excepted).

THE OAKLAND MOLE

This station differs from the two previously discussed, Crockett and Dumbarton, in that it is not a locality of strong or well-defined tidal currents, being rather in the backwash of the major currents which tend west of Goat Island. The average salinities here are lower than at any other station, except Crockett (see table 3), indicating that a portion of the ebb water from San Pablo Bay swings in here; and it may safely be assumed that a similar increment of ebb water from the southern arm of the bay is diverted to the east of Goat Island and mingles with the water coming from the northern arm. This situation is reflected in the variable condition of the water at the Oakland Mole, and the lack of any marked correlation with stages of the tide.

The maximum salinities here, as at Crockett, occur in general on a late flood or early ebb tide, when there is naturally the greatest concentration of ocean water; but the minimum salinities show no definite correlation with the tides, and probably depend on the local conditions mentioned.

The bottom salinities, with a few exceptions, are higher than the surface salinities, the average difference being 0.27 parts per 1000, in a depth at zero tide of twelve feet. As at Crockett and Dumbarton, a progressive increase in salinity is to be noted from the first to the last run.

The minimum temperatures, like the maximum salinities, occur on a late flood or early ebb tide, when the concentration of ocean water is greatest. The maximum bottom temperatures occur regularly on a late ebb or early flood, and the surface temperatures, although slightly more erratic, in general follow the same rule. The surface temperatures on three runs average 0.5° C. higher than the bottom temperatures.

The dissolved oxygen content of the water here is extremely variable, but higher in general than at any other locality in the bay.

There is a progressive increase in the oxygen content of the water through the month, the averages in cc. per liter for the three runs being, respectively, surface, 5.34, bottom, 5.39; surface, 5.53, bottom, 5.36; surface, 6.14, bottom, 5.86. There is little apparent correlation of dissolved oxygen with stages of the tide, although the minimum values occur most frequently on a late ebb.

The high oxygen content of the water at the Oakland Mole is difficult to explain, especially in view of the high index for dissolved hydrogen sulfide, which is usually an indication of oxygen deficiency (see p. 247). The results for hydrogen sulfide here are higher than at any other locality except Crockett. This is probably explained by the contamination of the water in this locality by the sewage of the city of Oakland. The Oakland Estuary, receiving the discharge of ten city sewers, opens about a quarter of a mile south of this station. To the northward, four sewers discharge directly into the bay, in the basin between the Southern Pacific and Key Route moles. One of these, at the foot of Seventh Street, discharges within three hundred yards of the point at which our samples were taken.

On the last run there appears to be a definite correlation between the hydrogen sulfide content of the water and tidal movements, the curve trending downward on each late flood, and upward on each ebb. On the two earlier runs, however, if there be any correlation at all, it is the reverse of the expected correlation.

It will be noted that the hydrogen sulfide steadily decreases from the first to the last run, while the oxygen, as we have stated, increases. Thus, to this extent at least, the expected reciprocal relation between the dissolved oxygen and hydrogen sulfide content of the water obtains.

The hydrogen-ion concentration of the water here, like the dissolved oxygen content, is unexpectedly high (average, surface, pH 7.94; bottom, pH 7.92), higher, in fact, than at any other locality except Fort Point. The results for this factor further exhibit on all three runs precisely the reverse of the expected correlation with tidal movements, being, with only two exceptions, highest on a late ebb or early flood, and lowest on a late flood or early ebb. This is another anomaly the explanation of which must await a more complete knowledge of the behavior of the tidal currents in this locality and of the conditions of the water in the areas immediately north and south.

THE SAN FRANCISCO FERRY BUILDING

Between the San Francisco waterfront and Goat Island strong tidal currents flow back and forth between the Golden Gate and the southern arm of the bay. Our station at the Ferry Building is on the border of this channel of strong tidal flow, and the results of observations here show in general a well marked correlation with tidal changes.

The salinity is regularly highest on the late flood or early ebb, and, with the exception of the beginning of the second run, lowest on a late ebb or early flood. Notwithstanding the slight depth of the water, only six feet at zero tide level, there is regularly a small difference between surface and bottom salinities, which averages 0.14 parts per 1000 for the three runs. The average salinities here are higher than at any other station in our series, except Fort Point. As at all stations thus far discussed, there is a progressive increase in salinity from the first to the last run.

The temperature bears the expected relation to tidal changes, and hence, in general, a reciprocal relation to the salinity, being lowest at the times of late flood or early ebb, and highest about the end of ebb and the beginning of flood. On each great ebb the temperature rises between one and two degrees centigrade in five hours, showing the influence of the warmer water flowing in from the southern portion of the bay. In spite of this fact, the average temperatures at the Ferry Building are lower than at any other locality except Fort Point.

The results for dissolved oxygen at this station are lower on the average than at any other station except Dumbarton, and are lower on the second run than on the first or third. The results are decidedly erratic and impossible to correlate with stages of the tide.

The results for dissolved hydrogen sulfide, on the other hand, exhibit a rather definite relation to tidal changes, being regularly highest on a late ebb or early flood tide, and, with the exception of the last run, lowest towards the end of the flood. The amount of dissolved hydrogen sulfide averages the same for surface and bottom on all three runs, and is greater on the second run than on the first or third. Thus, over a period of time, the expected reciprocal relation between dissolved oxygen and hydrogen sulfide is maintained.

The increase in hydrogen sulfide and the corresponding decrease in dissolved oxygen observed on the second run is attributed to sewage contamination. There are five sewer outlets along the San Francisco waterfront. One of these, at the foot of Howard Street, was about three hundred yards south of where our samples were taken. Another, at the foot of Jackson Street, was about five hundred yards north. The North Point outlet, which is the largest sewer of the city, discharges about one mile north of the Ferry Building. The sewage system of San Francisco is designed to take advantage of the strong ebb currents (see p. 209), which flush the waste from the city rapidly out to sea. During a neap tide period this flushing process is naturally less effective than during a period of spring tides, and the result is an increased concentration of hydrogen sulfide and a reduction in the amount of dissolved oxygen in the water along the San Francisco waterfront.

The results for hydrogen-ion concentration, like those for dissolved oxygen, are very erratic. If they be regarded as showing any correlation whatever with tidal changes, it is a reverse of the expected correlation, since the maximal values occur most frequently on a late ebb or early flood, and the minimal values on a late flood or early ebb. But the graph representing pH values at this locality is so irregular that this apparent correlation is considered to be of no significance.

FORT POINT

The results for salinity at Fort Point are approximately in accordance with the expectation, being highest near the end of flood and lowest near the end of ebb, with the exception of the last run, when a minimal surface salinity occurs on a late flood (10:26 A.M.). The graphs for salinity at this station (chart 5) also show, on the first run, a peak only slightly lower than the maximum for both surface and bottom at 6:02 P.M., just after low water slack; and on the last run a similar peak in the graph for bottom salinities occurs at 5:03 P.M., again just after low water slack. At the beginning of the first and last runs, the bottom as well as the surface salinity is unexpectedly low on a late flood tide.

Fort Point is furthermore the only station in our series at which there is not a progressive increase in salinity from the first to the last run. The average salinity results from the last run are higher than those for the first run but slightly lower than those for the second run.

It is unlikely that the omission of several determinations about the middle of the great ebb has any particular bearing on the case, as a mean weighted for the probable value of the missing samples differs from the simple mean by only a negligible amount. It will be noted that the range of salinities for the third run extends above and below that of the second, although the average of the latter is higher.

This is probably due to a difference in the tidal range on the two runs, the range on the third run being 2.1 feet greater than on the second. In a region of intermediate salinities an increase in the range of the tide will ordinarily raise the maximum and depress the minimum salinities in approximately the same degree, so that the average is little altered. But in a region of high salinities the maximum cannot be much increased by a strong flood, while the minimum will be decidedly lowered on a great ebb. At Fort Point, accordingly, which has the highest mean salinity of any station in our series, the average salinities will normally be lower on a spring tide than on a neap tide. In the present instance this factor is sufficient to offset the effect of the general increase in salinities throughout the bay.

The bottom salinities are higher than the surface salinities by an average amount of 0.14 parts per 1000, in water of a depth of eleven feet at zero tide level.

The lowest temperatures at Fort Point occur regularly on the late flood or early ebb, at approximately the periods of highest salinity. The maxima are less regular. On the first run both surface and bottom temperatures are highest at the beginning of observations, on a flood, although they drop rapidly toward the following slack water. The graphs show a similar anomaly at the beginning of the second run. In every case, however, the temperatures show a decided upward trend on the great ebb, which is in accordance with the expectation.

The results for dissolved oxygen at Fort Point are decidedly erratic, and it is doubtful that any real correlation exists with stages of the tide. While the minimum values for dissolved oxygen occur, with one exception, during the period from middle ebb to early flood, the maximum values, with but two exceptions, occur during the same period. The slight difference in the final averages between surface oxygen (5.34 cc. per liter) and bottom oxygen (5.32 cc. per liter) is regarded as significant, because the differences on the first run (surface, 5.19; bottom, 5.10, cc. per liter) and on the third run (surface, 5.55; bottom, 5.43, cc. per liter) are of greater magnitude. The smaller difference in the final averages is occasioned by the fact that, on the

second run, the expected relation between surface and bottom values is for some reason reversed (surface, 5.27; bottom, 5.43, cc. per liter).

It will be noted that the average values for dissolved oxygen increase slightly on each run.

The results for dissolved hydrogen sulfide are more regular than those for dissolved oxygen, being highest, with a single exception, on a late ebb or early flood, and lowest in general on a late flood or early ebb, except on the last run, when the values for dissolved hydrogen sulfide are low and constant during a long period, from early flood to late ebb, any variations occurring being too small to be detected by ordinary analytical methods.

The average amount of dissolved hydrogen sulfide in the water at Fort Point is lower than at any other station in the bay. The values are slightly higher on the first run than on the third, but higher on the second run than on either of the others.

The average Sörensen values at Fort Point are higher than those at any other station in the bay. It is therefore a logical assumption that the Sörensen value of the ocean water is higher than that of the bay water, and we should accordingly expect that the maximum values would be found on a late flood tide, and the minimum values on a late ebb. Such a correlation, however, is found only somewhat doubtfully on the second run, and not at all on the first and third. On these two runs, in fact, the data tend to show a reverse of the expected correlation, the graphs exhibiting a decided upward inflection on the great ebb. When this was observed on the first run it was thought that the determinations might be in error. But as the results on the third run are confirmatory, we are inclined to regard these data as authentic, although their significance is not clear.

The average Sörensen values on the first run are, if we consider surface and bottom values together, slightly higher than on the second run but slightly lower than on the third run.

It was expected that the conditions at Fort Point, in view of its location on the margin of the channel of the strong tidal currents between bay and ocean, would exhibit regular and marked correlations with the ebb and flow of the tide. It was therefore a matter of some surprise to find the results of our observations here extremely erratic, the variations noted in many cases showing little or no relation to the direction of flow of the major current at the time.

There is a slight possibility that sewage contamination may be in part responsible for the ecrhythmic variations in dissolved gases and

hydrogen-ion concentration at this station. The Fort Scott Mine Dock is about three miles west of the North Point sewer outlet, where the greater portion of the sewage of San Francisco is discharged. It is on the same side of the channel, and between the sewer outlet and the mouth of the harbor. For reasons later to be discussed (pp. 256 ff.), it is considered that the effect of sewage contamination in San Francisco Bay is usually rather promptly dissipated. But in view of the large volume of sewage discharged at the North Point outlet, it is possible that the condition of the water at Fort Point may be influenced to some extent by this factor. Such an assumption would explain the decrease in dissolved oxygen and Sørensen values observed soon after higher high water on both the first and the last runs, as the sewage is impounded by the high tide, and its greatest effect would be observable on an early ebb. By the middle of ebb the impounded sewage would be largely flushed out to sea, and the condition of the water in this region should approximate that of the bay water in general, which is the case so far as dissolved oxygen is concerned. The results for dissolved hydrogen sulfide and hydrogen-ion concentration are less in accord with this explanation.

Every higher low water is followed by a decrease in the amount of dissolved oxygen present. This may be explained by assuming that the small ebb is less effective as a flushing agent than the large ebb, so that, whereas the greatest concentration of contaminated water at Fort Point will occur early on a great ebb, it will occur late on a small ebb. At the beginning of the second run the usual sequence of the tides is altered, so that a higher high water which impounds a large volume of sewage, is followed by a small ebb instead of a great ebb; on this run there is an extraordinary drop in the values for dissolved oxygen about the end of the small ebb, correlated, as will be noted, with an upward inflection in the graph for hydrogen sulfide.

A further consideration which must be borne in mind in attempting to interpret the irregularities in the observed conditions at Fort Point is the probable nature of the local currents. The Fort Scott Mine Dock, which was the only available place to work in this locality, is not situated precisely on the Point, but in a partly sheltered cove just behind it (see fig. A, p. 206). Thus, although the dock projects some sixty yards from the shore, its outer end is still in the region of a kind of backwash from the main tidal currents. Furthermore, the currents here are rather erratic. It is stated in the United States Coast Pilot (1909, p. 71), "At the entrance they attain their greatest

velocity and are irregular in direction." Gilbert (1917, p. 71) observes that the flood tide, being opposed by the momentum of the ebb current, tends to flow in along the margins of the channel while in mid-channel the late ebb is still in motion.

In view of these local conditions, then, it is not surprising that the results of our observations fail to show an ideal correlation with the tides. To be properly representative of conditions in the Golden Gate, the water samples should be taken near the middle of the channel; this, however, was beyond the possibilities of our equipment.

DISCUSSION OF THE EFFECTS OF TIDAL CHANGES

The few investigations that have been made of the effect of tidal changes on the physical and chemical conditions of littoral waters have yielded somewhat conflicting results, which can probably be referred to differences in local conditions. Sumner *et al.* (1914), found that the salinity of water samples taken in San Francisco Bay on the early ebb is higher than that of samples taken on the early flood. This is in accord with our findings. Allen (1923) reports, on the basis of observations at the entrance of a small bay near San Diego, that the salinity is highest and that the pH values are lowest about low tide, while the reverse is true at high tide. This is similar to the condition found by us at Dumbarton, and is to be explained in the same way, the salinity of the water being increased by surface evaporation within the bay, while the alkalinity is somewhat decreased, presumably by a predominance of respiration over photosynthesis.

Powers (1920) reports that observations in Puget Sound "point to the probability that the water entering the Sound at high tide has a higher pH and a lower oxygen content than when flowing out at low tide," although he regards the evidence as inconclusive. Cameron and Mounce (1922) found no correlation of hydrogen-ion concentration with tidal movements in the Strait of Georgia (British Columbia). Atkins (1922) states that the condition of the water in Plymouth Sound "varies slightly with the state of the tide; a drop of pH 0.05 may sometimes be observed between high and low water." Allee (1923) found that, in inshore waters in the Woods Hole region, conditions which varied locally at low tide became more nearly uniform at high tide, although his investigations were not primarily concerned with tidal changes.

Our results in this connection, as presented in the foregoing pages, may be summed up in the statement that, of the five localities investigated, the three which border on strong tidal currents of well marked direction (Crockett, Dumbarton, and the Ferry Building) exhibit rather definite correlations of physical and chemical conditions with the ebb and flow of the tide, while those which border on currents of unknown or irregular direction (the Oakland Mole and the Fort Scott Mine Dock) show only a slight degree of correlation of the condition of the water with tidal changes.

It is difficult to make a general statement of the nature of the changes to be expected as a result of tidal movements, because of the differences that will occur with local conditions. Thus the maximum salinities occurred regularly near the end of flood at all stations except Dumbarton, where the ebb salinities were the higher, for reasons that have been mentioned. The temperature was everywhere lowest, with but rare exceptions, near the end of flood. The dissolved hydrogen sulfide was usually least near the end of flood, at all stations except Dumbarton, where no correlation of this factor with the tide was found, and at the Oakland Mole, where, on the last run only, the expected correlation was observed. The values for dissolved oxygen were regularly highest on a late flood at Dumbarton, while at Crockett they were usually highest about the end of ebb; at the other localities no correlation was observed, with the possible exception of the Oakland Mole, where the minimum values for dissolved oxygen occur most frequently on a late ebb. The pH values were highest about the end of flood at Crockett; the fragmentary data from Dumbarton indicate a similar correlation. The data from the other localities tend to show a reverse of this, the expected correlation. The significance of this fact has not been determined.

The tide is only one of a large number of factors determining the condition of the water at a given time and place. The sea is to be thought of as an immense complex of physical and chemical factors, many of them unknown or but little understood, the constant interaction of which produces changes of greater or less degree from hour to hour, and even from moment to moment. Thus it not infrequently occurs that the differences observed in a given factor between two successive hours are of greater magnitude than the differences observed between two successive tides. In the case of factors so unstable and so quickly affected by local conditions as dissolved gases and hydrogen-ion concentration, the isolated peaks are of little significance from the

hydrographic point of view. Conclusions can safely be based only on the general trend of conditions through a tidal cycle, or over a longer period of time.

For this reason the current ecological practice of taking high and low tide samples only, or of taking single samples at different localities, is open to considerable criticism. Results obtained from such isolated samples are extremely likely to be misleading. While it is usually not feasible to follow the conditions hour by hour, as undertaken in the present survey, at least a sufficient number of samples should be taken to insure that differences observed at different times and places are significant, and not merely the accident of an hour.

LOCALITIES COMPARED AS TO RANGE OF CONDITIONS

In table 4 the stations are ranked on the basis of the maximum range of conditions for any one run. In some cases, as an examination of the graphs will show, the greatest surface variation for a given condition occurs at one station, the greatest bottom variation at another. In such instances the ranking of the station is determined on the basis of the average of surface and bottom values. Thus in the case of dissolved hydrogen sulfide the greatest surface range for any one run (0.22 cc. per liter) occurs at the Oakland Mole, while the greatest bottom range (0.24 cc. per liter) occurs at Crockett. The average of surface and bottom maxima at each of these stations is the same (0.21 cc. per liter), so the two stations are given equal rank.

In table 5 the stations are grouped in the order of increasing range of conditions when the results for the entire period (three weeks) of investigation are considered. In other words, table 4 gives the ranking of the stations on the basis of the range of the conditions due primarily to the daily tidal changes, while table 5 indicates their rank on the basis of the range of conditions due rather to the major tidal differences of the month (spring and neap), and to such general factors as increasing temperature, diminishing run-off, and variations in the amount of sunshine.

In a few cases, as in that of the hydrogen-ion values at the Oakland Mole, it occurs that the greatest range on a single run is also the greatest range for the entire period, but as a rule the range of conditions through the month is greater than the range on any one run. Thus it comes about that the order of the stations is not quite the same on these two different bases of ranking.

Crockett, which ranks fourth on the basis of the diurnal range of conditions, takes second rank on the basis of the range of conditions over the three weeks of the investigation, owing largely, it will be noted, to a difference in the ranking of the Sørensen values. It might have been supposed that Crockett, in view of the continual interaction of river flow and tidal currents here, would exhibit the widest range in the condition of the water; but this is true only as regards the factors of salinity and dissolved hydrogen sulfide, other conditions remaining relatively constant.

TABLE 4

STATIONS RANKED ON THE BASIS OF THE MAXIMUM RANGE OF CONDITIONS FOR ANY ONE RUN

Station	Rank					Sum of ranks	Final rank
	Salinity	Tempera-ture	Dissolved O ₂	Dissolved H ₂ S	pH		
Oakland Mole	4	3	2	1.5	1	11.5	1
Ferry Building	2	2	3	3	2	12	2
Fort Point.....	3	1	1	4	4	13	3
Crockett	1	4	5	1.5	3	14.5	4
Dumbarton	5	5	4	5	5	24	5

TABLE 5

STATIONS RANKED ON THE BASIS OF THE MAXIMUM RANGE OF CONDITIONS FOR ALL RUNS

Station	Rank					Sum of ranks	Final rank
	Salinity	Tempera-ture	Dissolved O ₂	Dissolved H ₂ S	pH		
Oakland Mole.	2	2	1.5	2	1	8.5	1
Crockett	1	4	5	1	2	13	2
Ferry Building	3	2	3	3	3	14	3
Fort Point.....	4	2	1.5	4	4	15.5	4
Dumbarton	5	5	4	5	5	24	5

The widest range of conditions in general, on the basis both of diurnal variations and of variations over the entire period, is found at the Oakland Mole, which is so situated as to be affected more or less directly by currents from the upper bay, from the lower bay, and from the Golden Gate.

Conditions at the Ferry Building are less influenced by currents from the upper bay. The range of conditions here is consequently less than at the Oakland Mole, and only slightly greater than at Fort Point. At both the Ferry Building and Fort Point stations there is a wider diurnal range of conditions than at Crockett, but the range at the latter station is narrower over a period of time.

A question arises as to whether or not Dumbarton is properly ranked in these tables as being characterized by the least variable conditions, inasmuch as the limited range of conditions here is almost certainly due in part to the omission of the first run. But so nearly uniform are the conditions on the two runs that were made here that it appears unlikely that the results for an additional run would alter the ranking of the station.

It should be noted that the order of these stations on the basis of conditions through the month of July is probably not the order in which they would be ranked on the basis of conditions over a longer period of time. From what is known in general of conditions in San Francisco Bay throughout the year, it is considered probable that the greatest annual variation in physical and chemical conditions occurs in the upper bay, and that the least annual variation occurs in the vicinity of the Golden Gate.

TURBIDITY

As no correlation was observed between the turbidity of the water and any other condition studied, it is convenient to give here a brief separate presentation of the results for this factor. The data are not wholly satisfactory, owing to the limited number of standards (see p. 222) which it was feasible to carry. When an exact match could not be obtained, the turbidity was expressed in terms of the nearest standard followed by a plus or minus sign. Except at Crockett and Dumbarton, it commonly occurred that the turbidity was less than our lowest standard (20), although the water was, of course, not perfectly clear. In such cases the turbidity was indicated as 20-, or "negligible," at the discretion of the observer. This is palpably an unsatisfactory procedure, but the results are considered still to possess at least a relative value.

It will be observed (table 6) that the highest turbidity occurs at Crockett, largely as a result of the silt carried down by the rivers. The turbidity of the bottom water here, in a depth of 31 feet, is rather more than twice as great as the surface turbidity, indicating the rapid

settlement of this suspended débris, which causes so much difficulty in keeping open navigable channels in the upper bay. Gilbert (1917, p. 35, pp. 91 ff.) considers that the greater portion of the river-borne débris is deposited by settlement and flocculation in Suisun and San Pablo bays and the connecting strait, that a relatively small portion is deposited in the middle bay, and that hardly more than a negligible amount is carried out through the Golden Gate. This statement is borne out by our results.

TABLE 6
RESULTS FOR TURBIDITY
Expressed as parts of silica per million

Station	Maximum			Minimum			Average		
	First run	Second run	Third run	First run	Second run	Third run	First run	Second run	Third run
Crockett:									
Surface	400	200+	200	50	100-	20	240	110	110
Bottom	1000	800	400+	100	100-	100-	490	260	270
Dumbarton:									
Surface		200-	200+		100-	50		120	180
Bottom		200+	400-		100-	100		130	210
Oakland Mole:									
Surface	50	20-	(¹)	20-	(¹)	(¹)	20	(¹)	(¹)
Bottom	50	20-	(¹)	20-	(¹)	(¹)	20	(¹)	(¹)
Ferry Building:									
Surface	50	20	20-	20-	(¹)	(¹)	30	20-	(¹)
Bottom	50	20+	20-	20-	(¹)	(¹)	30+	20-	(¹)
Fort Point:									
Surface	20-	20	(¹)						
Bottom	20-	20	(¹)						

¹ Negligible

The high turbidity at Dumbarton is attributed to the action of rapid tidal currents in stirring up bottom material, the bottom in this portion of the bay consisting almost entirely of mud (see Sumner *et al.*, 1914, pl. 5). Although the depth is greater than at Crockett, the difference between surface and bottom turbidities is slight, as we do not have here the factor of flocculation which operates when fresh-water suspensions come in contact with salt water.

As the channel widens north of Dumbarton, however, this suspended matter must settle out more or less rapidly, as very little of it reaches the middle bay. The turbidity at the Ferry Building was small, and at the Oakland Mole still less. The very slight turbidity that was observed at Fort Point appeared to be due largely to the

plankton content of the water, rather than to the presence of suspended débris.

It should be noted that these data probably represent very nearly the minimal turbidities of the year. During the period of maximal run-off the turbidity of the water in all portions of the bay must be very much greater.

INTERRELATIONS OF DISSOLVED GASES AND HYDROGEN-ION CONCENTRATION

Normal sea water is a slightly alkaline medium, having a moderate buffer effect which is dependent principally on the carbonate equilibrium, i.e., the equilibrium between dissolved carbon dioxide and the carbonates, and more especially the bicarbonates, of calcium and magnesium, which are formed in sea water in the presence of carbon dioxide (cf. Murray and Hjort, 1912, pp. 175 ff.; Clark, 1922, p. 344; Atkins, 1922, p. 732, etc.). Thus the most important single factor influencing the hydrogen-ion concentration of sea water is the carbon dioxide tension. So definite is the relation of these two factors that McClendon (1917) has constructed a graph of the relations of hydrogen-ion concentration, carbon dioxide tension, and titrable alkalinity of sea water, from which it is possible to calculate any of these three factors if the other two be known.

It is well known that carbon dioxide tension is closely related to the photosynthesis carried on by the phytoplankton and other forms of marine vegetation. As the same process which removes carbon dioxide from the water releases a proportional amount of oxygen, these two gases bear very roughly a reciprocal relation in sea water—a relation which is extremely variable, owing to the fact that the amounts of both gases in solution are influenced by a number of factors besides photosynthesis. These relations have been adequately discussed in recent papers by Atkins (1922, 1923) and Helland-Hansen (1923), where full bibliographies will be found. In view, then, of the fact that both Sørensen values and values for dissolved oxygen are inversely related to the carbon dioxide tension, we are led to expect that high oxygen values will be correlated with high pH values, and low oxygen values with low pH values (cf. Palitzsch, 1912; Moore *et al.*, 1915; Osterhout and Haas, 1918).

Dissolved hydrogen sulfide of itself has very little effect on the hydrogen-ion concentration of sea water, in the first place because it

ordinarily occurs in very small amounts, in the second place because the gas is only slightly dissociated in solution, and in the third place because sea water is buffered by the carbonate equilibrium, as mentioned above. Thus, theoretically the presence of even considerable amounts of dissolved hydrogen sulfide should not appreciably alter the alkalinity of sea water, and it has been the observation of the authors that stagnant aquaria black with bacteria and giving off a strong odor of sulfuretted hydrogen may have a Sörensen value only slightly below that of fresh sea water.

Ordinarily, however, there is an indirect relation between the pH values and dissolved hydrogen sulfide, due to the fact that the latter is unable to persist long in appreciable quantities in the presence of dissolved oxygen, being converted to molecular sulfur. Thus a high pH value, usually associated with a high oxygen content, is accordingly indicative in general of a low value for dissolved hydrogen sulfide.

It becomes obvious from an inspection of the charts that there is no immediate correlation between the hydrogen-ion concentration and dissolved oxygen or hydrogen sulfide. A relation of these factors noted in the case of any given sample may be quite reversed in a sample taken an hour later. If, however, instead of comparing the hourly conditions of the individual peaks, we turn attention to the general results as set forth in table 3, we find that, over a period of time, the expected relations among these factors are usually in evidence.

Thus at Crockett the dissolved oxygen content of the water is higher on the first run than on the second, but lower than on the third, the averages for the three runs being, respectively, 5.42, 5.23, and 5.57 cc. per liter (for convenience in discussion surface and bottom conditions are averaged together). As regards Sörensen values, the same relation is found, the figures comparable with the above being pH 7.67, pH 7.59, and pH 7.76 respectively. The values for dissolved hydrogen sulfide (0.24, 0.16, and 0.09 cc. per liter for the three runs) show a steady decrease from first to last, instead of an increase on the second run, as would ordinarily be expected from the lower oxygen and pH values. This is not actually an anomaly, however, since, while a high oxygen content will rapidly reduce the amount of hydrogen sulfide present, a low oxygen content will not, of itself, increase the dissolved hydrogen sulfide.

The results from Dumbarton are too meager to be of significance in this connection.

At the Oakland Mole there is a progressive increase in the amount of dissolved oxygen (5.37, 5.45, and 6.00 cc. per liter for the three runs), a corresponding increase in the Sörensen values (pH 7.90 pH 7.93, and pH 7.97), and a progressive decrease in the amount of dissolved hydrogen sulfide (0.21, 0.15, and 0.10 cc. per liter).

At the Ferry Building the dissolved oxygen content of the water is decidedly lower on the second run than on the first, but higher on the third run than on either, the figures being 5.29, 5.08, and 5.35 cc. per liter, respectively. In this case the hydrogen sulfide shows the expected inverse correlation with the dissolved oxygen, the averages being 0.13, 0.17, and 0.09 cc. per liter for the three runs, but the Sörensen values show a progressive increase from the first to the last run (pH 7.83, pH 7.86, pH 7.93).

At Fort Point the oxygen values increase progressively from first to last (5.15, 5.35, and 5.49 cc. per liter); the values for dissolved hydrogen sulfide are higher on the second run than on the first, but are lowest on the third run (0.07, 0.10, and 0.06 cc. per liter, respectively); the Sörensen values are a very little lower on the second run than on the first, and a little higher on the third run than on the first (pH 7.95, pH 7.94, pH 7.96). It cannot be said that any definite correlation appears among these data, except that the results for pH and hydrogen sulfide are inversely related, a fact which in this instance is probably of no significance. It should be noted, however, that here, as, in fact, at all of our stations (Dumbarton excepted), on the last run the oxygen and pH values are higher, and the hydrogen sulfide values lower, than on either of the preceding runs.

The general averages of dissolved oxygen, hydrogen sulfide, and pH values on each run, for four stations (Dumbarton being excluded), are given in table 7. There is seen to be a direct correspondence between the oxygen and pH values, and practically the expected inverse correlation with dissolved hydrogen sulfide.

Thus the statement appears justified that, in general, a high Sörensen value is associated with a relatively large amount of dissolved oxygen, and a proportionately small amount of dissolved hydrogen sulfide, although these relations are so uncertain that the Sörensen value alone cannot be regarded as a satisfactory index of the relative concentrations of these gases.

TABLE 7

AVERAGE VALUES FOR DISSOLVED OXYGEN, DISSOLVED HYDROGEN SULFIDE, AND
HYDROGEN-ION CONCENTRATION ON EACH RUN
(Averages for four stations, Dumbarton being excluded)

	O ₂ cc. per liter	Hydrogen-ion concentration pH	H ₂ S cc. per liter
First run.....	5.31	7.84	0.16
Second run	5.28	7.83	0.15
Third run.....	5.60	7.90	0.08

In connection with the buffer action of sea water, it is interesting to note that the minimum alkalinity found at any of our regular stations was pH 7.50, at Crockett, while the maximum alkalinity was pH 8.16, at the Oakland Mole, thus giving a total range of only 0.66 Sörensen unit for the entire bay, with the exception of the special conditions prevailing in immediate proximity to the mouths of sewers (*vide infra*). This indicates a remarkable uniformity of hydrogen-ion concentration, in spite of widely diverse conditions of depth, salinity, temperature, and dissolved gases, affording a striking illustration of the suitability of sea water as a biological medium, corresponding in the economy of many marine invertebrates to the blood of vertebrate forms.

EFFECT OF METEOROLOGICAL ON HYDROGRAPHIC CONDITIONS

The condition of sea water is affected both directly and indirectly by meteorological conditions. Among the direct effects we may number temperature, salinity as affected by evaporation, and the mechanical effect of wind in agitating the water, thus altering in some degree the dissolved oxygen content, and causing more or less extensive movements of the water. The great equatorial drift currents are attributed to the trade winds, and various more local surface or vertical currents are caused by wind, an extensive movement of surface water under appropriate conditions causing an upwelling of the water from beneath (McEwen, 1916, p. 277). The indirect effects of meteorological conditions on the condition of sea water include the influence of light and temperature on the rate of photosynthesis, and the influence of temperature and, in less degree, of barometric pressure, on the solubility of gases in sea water.

The relation of water temperatures in San Francisco Bay to local air temperatures has been discussed by Davidson (1885), and more recently by Sumner *et al.* (1914, p. 50). The air temperatures are, in general, higher than the water temperatures during the warmer months of the year, and lower than the water temperatures during the colder months; but the differences are relatively small, and the mean annual temperatures of air and water are approximately the same. Thus Sumner *et al.* (*loc. cit.*), found the temperature of San Francisco Bay for one year to average 12.91° C., which corresponded to an average air temperature of 13.53° C. for San Francisco and Berkeley. From the graphic presentation of Sumner's data, which is reproduced here (fig. D), it will be seen that our observations were made at the period of maximum water temperatures, though not of maximum air temperatures, for the year.

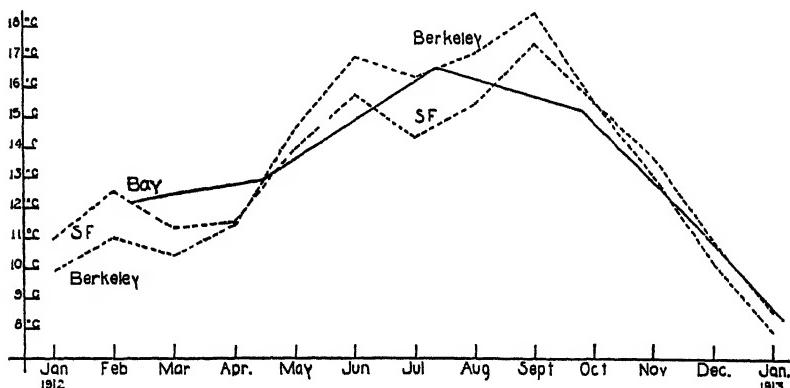


Fig. D. Relations between local air and water temperatures. The continuous line represents the annual temperature variations for the bay as a whole. The two broken lines are based upon the monthly means for air temperature at San Francisco and Berkeley during the year covered by the hydrographic observations. (From Sumner *et al.*, 1914.)

Air temperatures were recorded hourly during the present investigation by means of an ordinary centigrade thermometer hung in the shade. As no relation is to be observed between the minor fluctuations of air and water temperatures, it is not considered worth while to report the hourly air temperatures. In general the air and water became isothermal for a short time in the morning, between 9:00 o'clock and noon, after which the air temperature rose several degrees above the water temperature during the afternoon, then declined towards an evening isothermal period, sometime between sundown and midnight.

These relations were much modified by changes in the temperature of the water due to tidal currents, as discussed in a preceding section.

The general relations between air and water temperatures at the different stations can be noted in table 8. The water temperatures given are the averages of surface and bottom values.

TABLE 8
COMPARISON OF AVERAGE AIR AND WATER TEMPERATURES FOR EACH RUN
AT EACH STATION
(Temperature in ° C.)

Station	Oakland Mole		Ferry Building		Fort Point		Dumbarton		Crockett	
	Air	Water	Air	Water	Air	Water	Air	Water	Air	Water
First run	15 1	17 0	15 4	14 8	14 4	13 4			16 4	18 5
Second run	15 7	17 7	16 7	15 3	14 7	14 4	(16 0)	(20 1)	19 3	19 3
Third run	15 2	18 7	15 8	15 7	13 4	14 6	(15 8)	(21 1)	22 4	19 5

It will be noted that, in general, the air temperatures are somewhat lower on the third run than on the second run, although the water temperatures increase regularly from the first to the last run at each station. The high value for air temperature on the last run at Crockett probably represents an unusual condition. This run began on the afternoon of July 27, which, according to the report of the Weather Bureau Station at San Francisco, was the warmest day of the month. The air temperatures recorded for Dumbarton are not representative, as both runs here included the entire night, but only a part of the day. The temperatures for the entire day would doubtless increase the average by two or three degrees.

In the following summary of air and water temperatures (table 9), the data for four stations are averaged together, Dumbarton being excluded for the reason just mentioned. The number of hours of sunshine are from the records of the San Francisco station of the U. S. Weather Bureau. In each case the value given is the total number of hours of sunshine for a six-day period, as each of our three periods of investigation included four full days, and a part of one day preceding, and of one day following.

It is interesting to note that the average water temperature is in each case slightly higher than the average air temperature, indicating that insolation is a more important factor in warming the water than is the actual temperature of the air.

TABLE 9

AVERAGE AIR AND WATER TEMPERATURES AND CORRESPONDING NUMBER OF
 HOURS OF SUNSHINE DURING ALL RUNS
 (Averages for four stations, Dumbarton being excluded)

	Average air temperature °C	Average water temperature °C	Hours of sunshine (San Francisco)
First run	15.3	15.9	54.3
Second run	16.6	16.7	66.4
Third run	16.7	17.1	79.3

The joint influence of increasing temperature and sunshine is reflected in the increase in values for dissolved oxygen from the earlier to the latter part of the investigation, which has been pointed out above (table 7). It has been shown by Blackman (1905) and Blackman and Smith (1911) that, within certain limits, an increase in temperature of 10° C will about double the rate of photosynthesis, which also varies directly with the illumination and the carbon dioxide tension.

We should expect also a day and night variation in amount of dissolved oxygen, owing to the fact that photosynthesis ceases at night, while oxygen continues to be used in the respiration of both animals and plants. Jacobsen (1912) has reported an average dissolved oxygen content of surface water in the North Atlantic, in June, of 5.9 cc. per liter during the day, and 5.34 cc. per liter at night (in September the diurnal variation was found to be of smaller amplitude). McClendon (1918) found that the dissolved oxygen in sea water near the Tortugas increased fairly regularly from early morning to late afternoon, although secondary variations were caused by local currents.

In the present investigation it has been difficult to separate the effect of photosynthesis from the changes in dissolved oxygen content of the water due to tidal currents, which have been discussed in a preceding section. Variations in dissolved oxygen due to the latter cause, more or less obscure any regular day and night rhythm; but in some cases an increase in dissolved oxygen observed during the afternoon can be attributed with some degree of certainty to the immediate effect of photosynthesis.

It has been noted (p. 229) that on the second run at Crockett the expected relation of dissolved oxygen to tidal changes is practically reversed. In this instance, the surface values for dissolved oxygen,

which should reflect most immediately the effect of temperature and sunshine on photosynthesis, decline from a maximum at 6:15 P.M. (the beginning of the run) to a minimum at 4:43 A.M., then ascend more or less regularly until the completion of the run at 2:03 P.M., thus showing practically a perfect correlation of dissolved oxygen with light and temperature. The failure to observe any effect of tidal currents in this instance is explained at least in part by the fact that the range of the tide on this run was only about two-thirds as great as on the two other runs at Crockett.

The bottom values do not show a similar correlation; but it is probable that little or no photosynthesis is carried on at a depth of 31 feet, in water as turbid as commonly found here (see table 6).

At Dumbarton any effect of photosynthesis on the dissolved oxygen content of the water is obscured by the greater changes due to tidal currents. At the Oakland Mole in just one instance can an apparent direct effect of photosynthesis on the amount of dissolved oxygen be noted. This occurs on the last run, when, at 3:46 P.M., the highest surface value for dissolved oxygen obtained anywhere in the bay was recorded, and at 4:54 P.M. the highest value for bottom oxygen was found. These values coincide in occurrence, respectively, with the highest surface and bottom temperatures found at the Oakland Mole, which is in accord with the fact mentioned above, that the rate of photosynthesis increases rapidly with rising temperatures. These maximal oxygen values represent a condition of supersaturation, at the corresponding salinity and temperature values, of 125 per cent and 120 per cent for surface and bottom, respectively. As this is the only instance of actual supersaturation found during the investigation, there seems little reason to question that the condition is the immediate result of increased photosynthesis under the favorable conditions prevailing at this time.

The results for dissolved oxygen at the Ferry Building do not show any satisfactory correlation with the presumed rate of photosynthesis as affected by light and temperature. At Fort Point, on the first run the highest surface value for dissolved oxygen occurs at 4:59 P.M., and on the last run the highest surface value occurs at 3:58 P.M. As both of these peaks occur about the end of a small ebb, which is not in accordance with any expected relation to the tides, it appears legitimate to attribute the increase in dissolved oxygen directly to photosynthesis.

Accordingly it may be said that the results for three of the five stations show a slight tendency towards a day and night rhythm in amount of dissolved oxygen, which, however, is more or less obscured by the changes due to tides and local currents.

It is interesting to consider the results for dissolved oxygen in terms of percentage of saturation. Saturation of a liquid with a gas is defined as the amount of the gas that will be absorbed by the liquid under given conditions of temperature and pressure. Since, however, absorption from the atmosphere is only one of a number of factors affecting the dissolved oxygen content of sea water, conditions of subsaturation or supersaturation are commonly encountered, absolute saturation being merely a coincidence.

The average oxygen content of San Francisco Bay waters is somewhat below the amount required to constitute saturation. The single instance of supersaturation encountered at the Oakland Mole has been mentioned. Conditions of approximate saturation were found at Fort Point in the case of the surface value for oxygen at 4:59 P.M. on July 12, and in the case of the bottom value at 4:46 P.M. on July 18. Elsewhere, and at other times, the dissolved oxygen has been below saturation, but usually not much below.

TABLE 10

AVERAGE VALUES FOR DISSOLVED OXYGEN AT EACH STATION CALCULATED AS PER CENT OF SATURATION

Station	Average O ₂ cc per liter		Normal absorption of O ₂ cc per liter (after Fox)		Average O ₂ per cent of saturation	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
Crockett	5.47	5.34	6.14	6.08	89	88
Dumbarton	4.68	4.55	5.55	5.55	84	82
Oakland Mole	5.67	5.51	5.83	5.88	97	94
Ferry Building	5.26	5.21	6.04	6.06	87	86
Fort Point	5.34	5.32	6.11	6.11	87	87

In table 10 are given the average values for dissolved oxygen at each station and the per cent of saturation each represents. The latter was calculated (partly by interpolation) from the tables of Fox (1907), reproduced by Helland-Hansen (1923), using the corresponding average values for temperature and salinity as given in table 3. No correction has been made for variations in barometric pressure.

The tables of Fox assume a pressure of 760 mm. The mean atmospheric pressure during the month of July was 760.9 mm. (San Francisco station U. S. Weather Bureau). The normal absorption of oxygen by sea water decreases with increasing temperature and increasing salinity. Thus it comes about that, although the average amount of dissolved oxygen at Dumbarton is considerably less than at the Ferry Building, the per cent of saturation is nearly the same, because of the higher temperature at Dumbarton. When we compare Crockett and the Ferry Building, we find that both the average oxygen and the per cent of saturation are nearly the same for the two stations, the lower salinity at Crockett, which would increase the absorption coefficient for oxygen, being about balanced by the higher temperature, which would decrease the normal absorption.

This table shows a fair uniformity in what may be termed the relative amounts of dissolved oxygen throughout the bay, the water at the various stations being, on the average, more than four-fifths saturated with oxygen.

It is interesting to note also that the per cent of saturation increases through the month. In table 11 the average results for dissolved oxygen on each run (mean surface and bottom values at four stations averaged together, Dumbarton being omitted) are converted to per cent of saturation. While the absolute amount of oxygen is a little less on the second run than on the first, as stated above (table 7), it will be seen from this table that the relative amount of oxygen is slightly greater on the second run, so that, considered in terms of per cent of saturation, there is really a progressive increase in dissolved oxygen through the period of the investigation, corresponding to the increase in temperature and amount of sunshine (table 9).

TABLE 11
AVERAGE VALUES FOR DISSOLVED OXYGEN ON EACH RUN CALCULATED IN
TERMS OF PER CENT OF SATURATION
(Averages for four stations, Dumbarton being excluded)

	Average temperature °C.	Average salinity parts per 1000	Average O ₂ cc. per liter	Normal absorption of O ₂ cc. per liter	Average O ₂ per cent of saturation
First run .	15.9	24.67	5.31	6.11	87
Second run .	16.7	25.71	5.28	6.00	88
Third run .	17.1	26.71	5.60	5.93	94

Of other effects of meteorological conditions on the condition of the bay water, the increase in salinity in the southern portion of the bay due to evaporation has already been discussed (p. 231). Wind is a factor the effect of which we have not attempted to investigate. No heavy winds occurred during the month, but a moderate wind from the ocean was characteristic of a part of nearly every day. Wind is an important agent in increasing evaporation, and in causing more or less extensive movements of the water. As a direct means of aeration its effect is probably slight, on account of the slow diffusion of atmospheric oxygen into sea water, especially when the latter is already nearly saturated. Under conditions of supersaturation, agitation of the water by wind may cause the excess oxygen to be given off into the atmosphere, although, as stated by Mellor (1922, p. 369), water is easily supersaturated with oxygen, and the excess is given off with difficulty.

EFFECT OF SEWAGE CONTAMINATION

Contrary to the expectation of the authors at the beginning of this investigation, contamination by sewage and industrial wastes has appeared to be a factor of little importance in its effect on the condition of the water in San Francisco Bay. The relatively large amount of dissolved hydrogen sulfide found at the Oakland Mole is attributed (p. 234) to contamination by sewage of the city of Oakland. It is probable also that the still higher values for dissolved hydrogen sulfide at Crockett (p. 230) are due to sewage carried down by the San Joaquin and Sacramento rivers, as the results indicate rather definitely that the river water contains more hydrogen sulfide than the bay water. The increase in amount of hydrogen sulfide, and decrease in amount of oxygen, observed on the second run at the Ferry Building (p. 236) is attributed to contamination, the neap tide being regarded as less effective than a spring tide in carrying the sewage of San Francisco out to sea. It has been thought also that certain of the erratic results for dissolved gases and hydrogen-ion concentration noted at Fort Point (p. 239) may have been due to sewage pollution. But in general the chemical effect of such contamination was nearly negligible, even the maximum values for dissolved hydrogen sulfide being very small, while the values for dissolved oxygen and pH were correspondingly high.

In order to throw further light on this matter, it was decided to make a special investigation of conditions near the mouth of a sewer,

to determine the effect of pollution on the water immediately proximal to the sewer outlet, and the rapidity with which such effect is dissipated with distance. The locality selected for this purpose was the Channel Street canal, San Francisco. This canal, about fifteen hundred yards long and fifty yards in width, joins the bay in the so-called "China Basin," near pier 42 (see fig. E). The only flow of water is that produced by the rise and fall of the tide. At its upper end the canal receives the discharge of the Channel Street sewer. This sewer is designed primarily for storm water overflows, but is temporarily carrying some human sewage, and has in the past carried a great deal more.

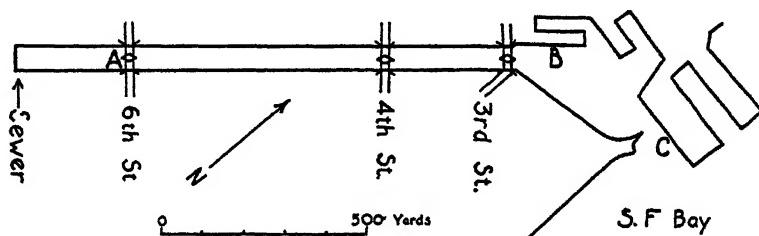


Fig. E. Map of Channel Street canal, San Francisco, showing localities (A, B, and C) at which water samples were taken.

At the upper end of the canal there is an entire absence of marine life, the bottom of the channel and the piling supporting the structures along its shores being covered with a coating of black sludge, which on the bottom reaches a depth of many feet. This condition is probably due largely to factors no longer operative, as in past years a much larger amount of human sewage was discharged into the canal than is the case at the present time. Nevertheless, the upper end of the canal presents conditions of extreme pollution, the water being discolored and bubbles of gas being continually given off from the decomposing sludge on the bottom. These conditions become progressively less apparent with increasing distance from the mouth of the sewer, and at the lower end of the canal the visible effects of pollution disappear and some marine organisms are able to survive. The wood-boring organism *Bankia setacea* has been found in piling along the sides of the canal approximately one hundred yards from its junction with the bay.

Water samples were taken at low water slack on August 15, 1923, and at high water slack on the following day, at each of three localities along the canal: one at the upper end, about 200 yards from the mouth

of the sewer (station A); one near the lower end, about 100 yards from the bay (station B); and one on pier 42, about 100 yards outside the entrance to the canal (station C). (Pier 42 and the structures shown opposite it in figure E are wharves built on piles, and do not represent the actual boundaries of the channel in this region.) As the depth of the water at the upper end of the canal was only about one foot at low tide, and about six feet at high tide, one sample was considered sufficiently representative of both surface and bottom conditions. At stations B and C, where the depths at low tide were, respectively, thirty and forty feet, separate surface and bottom samples were taken. The results are shown in table 12.

TABLE 12
CONDITIONS AT THE MOUTH OF THE CHANNEL STREET SEWER

	Salinity parts per 1000		Dissolved oxygen cc per liter		Dissolved hydrogen sulfide cc per liter		Hydrogen-ion concentration pH	
	Low water	High water	Low water	High water	Low water	High water	Low water	High water
Station A	25.77	30.39	(1.60)	2.61	12.09	0.08	7.09	7.42
Station B:								
Surface	30.23	30.68	3.75	3.69	0.08	0.10	7.50	7.48
Bottom	30.73	31.29	4.38	6.17	0.08	0.08	7.50	7.67
Station C:								
Surface	30.53	30.53	6.14	5.69	0.10	0.10	7.61	7.77
Bottom	30.61	31.78	6.07	6.35	0.10	0.10	7.74	7.76

The low water value for dissolved oxygen at station A was determined from a separate sample taken on August 17, as an error was made in the titration of the sample taken on August 15, through failure to allow for the unexpectedly large amount of hydrogen sulfide present. This determination is therefore not directly comparable with the others, but is interesting nevertheless as an indication of the extremely low oxygen content of the water here at low tide. The height of low water on August 15 was 0.5 feet; of high water on August 16, 5.7 feet; and of low water on August 17, 1.5 feet.

At station A, at low tide, the conditions of extreme pollution prevailing are reflected in the low oxygen content of the water, and the low pH value, which is barely on the alkaline side. The extremely

high value for hydrogen sulfide is probably greatly in excess of the actual amount of the dissolved gas present, representing in part various organic (reducing) substances derived from the sewage and the decomposing sludge on the bottom. The sample was decidedly discolored, probably by bacteria. The lowered salinity (25.77 parts per 1000, compared with an average of 30.53 for stations B and C on this low tide) indicates that the water at the upper end of the canal at this time was diluted with about one-fifth of its volume of fresh water coming in from the sewer. That is, one-sixth or more of the sample taken was actually sewage. None of this was storm water, as our samples were taken during the dry season. We did not attempt to estimate the amount of organic matter present; but the sewage was naturally more concentrated at this time than at other seasons of the year.

At high tide the water at station A becomes diluted with several volumes of water from the lower end of the canal and the bay. The salinity is increased until it shows scarcely any effect of dilution with sewage. The oxygen and pH values are raised, though they still remain decidedly low. The most decided change is in the result for hydrogen sulfide, which on the high tide is surprisingly low. In this connection it should be remarked that the sample at high tide was taken about three feet from the bottom, and did not show any discoloration by sulfur bacteria, as was the case in the sample taken at low tide. Nevertheless, this value is unexpectedly small.

At station B the effect of pollution is still evident in the low oxygen and pH values. The salinity is rather above the average for this part of the bay, while the hydrogen sulfide is practically negligible. The fact that the surface values for oxygen are lower than the bottom values, which is contrary to the general rule, indicates that the sewage contaminated water tends to flow out on top of the more saline bay water. This is also shown in the pH values at high tide.

At station C, 100 yards outside the entrance to the canal, the chemical effects of pollution have almost or altogether disappeared. The salinity and dissolved oxygen values are higher, and the hydrogen sulfide values lower, than is the average for this part of the bay (judging by our results at the Ferry Building). The Sörensen values are lower than the average, but this may only very doubtfully be attributed to the effect of sewage contamination.

These data are very fragmentary, and conclusions based upon them are open to the criticism, already voiced by the authors (p. 242), of being deductions drawn from an insufficient number of samples. The results for hydrogen sulfide in particular are anomalous, being remarkably high at station A at low tide and surprisingly low in all other cases. It is not believed that this is due to errors in analysis; but a larger number of samples might show a different condition. The data are merely presented for what interest or value they may possess. They afford some index to the conditions actually prevailing in the mouth of a sewer, and, with the exception of those for hydrogen sulfide, the results are in accordance with the expectation—the salinity, dissolved oxygen, and pH values all being lowest at station A, nearest the mouth of the sewer, and showing a progressive increase with distance from the sewer.

These data, together with the general results of our survey, point to the conclusion that the chemical effect of the sewage at present being discharged into San Francisco Bay is inconsiderable and is quickly dissipated. This is due in part to the absence of any great amount of industrial wastes in the sewage, human sewage being much less objectionable biologically than chemical wastes; but the principal factor is the constant interchange of water between bay and ocean, with strong tidal currents which occasion rapid dilution of the sewage, facilitate oxygenation of the water, and prevent stagnation and putrescence even in localities where a considerable volume of sewage is emptied into the bay.

More serious than the chemical effect on the water is the simple mechanical effect of the solid matter which accumulates at the mouth of a sewer. Of the total amount of solids present in a sewage, according to the estimate of Winslow and Phelps (1906), from 60 to 70 per cent is in solution, either true or colloidal. Solids in the colloidal state are quickly precipitated on contact with salt water and settle to the bottom. There is thus a gradual accumulation of sludge on the bottom of the bay in the immediate vicinity of a sewer outlet, which renders the bottom entirely untenable for any life except the bacteria of decay. The areas of bottom thus affected in San Francisco Bay are inconsiderable at present; but it is to be feared that they will constantly increase as growing populations cause larger volumes of sewage to be emptied into the bay. It is unhappy to contemplate that the portion of the bay which is likely to be most speedily affected by accumulating sludge is the middle region, which Packard (1918) has shown to support the richest molluscan fauna.

SUMMARY

1. A study has been made of the salinity, temperature, turbidity, dissolved oxygen, dissolved hydrogen sulfide, and hydrogen-ion concentration of the water at five selected localities on San Francisco Bay, in relation to tidal changes, and also in relation to meteorological conditions and sewage contamination. The observations were carried on during a period of about three weeks, in July, 1923.

2. Surface and bottom water samples were taken at approximately hourly intervals through three twenty-hour (minimum) periods about one week apart, representing two spring tides and the intervening neap tide, at each of the following stations: Fort Scott Mine Dock (near Fort Point), the San Francisco Ferry Building, the Oakland Southern Pacific Mole, and Crockett, on Carquinez Strait. A supplementary study, less complete, was made at Dumbarton, near the southern end of the bay.

3. Of the five localities investigated, the three which border on strong tidal currents of well marked direction (Crockett, Dumbarton, and the Ferry Building) exhibit rather definite correlations of physical and chemical conditions with the ebb and flow of the tide, while those which border on currents of unknown or irregular direction (the Oakland Mole and the Fort Scott Mine Dock) show only a slight degree of correlation of the condition of the water with tidal changes.

4. The maximum salinities occurred regularly near the end of flood at all stations except Dumbarton, where the ebb salinities were the higher, owing to rapid evaporation in the shallow southern reaches of the bay.

5. The temperature was everywhere lowest, with but rare exceptions, near the end of flood.

6. The dissolved hydrogen sulfide was usually least near the end of flood, except at Dumbarton, where no correlation of this factor with the tide was found, and at the Oakland Mole, where, on the last run only, the expected correlation was observed.

7. The values for dissolved oxygen were regularly highest on a late flood at Dumbarton, while at Crockett they were usually highest about the end of ebb; at the other localities no correlation was observed, with the possible exception of the Oakland Mole, where the minimum values for dissolved oxygen occur most frequently on a late ebb.

8. The Sörensen values were uniformly highest near the end of flood at Crockett. The meager data from Dumbarton indicate a

similar correlation. The data from the other localities either show no correlation, or tend to exhibit a reverse of the expected correlation.

9. The average conditions at each station and the ranking of the stations on the basis of range of conditions are shown in tabular form (tables 3, 4, and 5).

10. The turbidity of the water was found to be greatest at Crockett and least at Fort Point. No correlation was observed between this and any of the other factors studied. The results for turbidity are shown in table 6.

11. Over a period of time, the Sörensen values were found to vary directly with the amount of dissolved oxygen; the dissolved hydrogen sulfide bore an inverse relation to these two factors.

12. The surface water, as compared with the bottom water, even in depths of only a few feet, was characterized by a lower average salinity, higher temperature, larger amount of dissolved oxygen, and higher Sörensen value. The average amount of dissolved hydrogen sulfide was about the same in both surface and bottom waters.

13. During the period of the investigation there was a progressive increase in the per cent of saturation with dissolved oxygen, correlated with a progressive increase in temperature and number of hours of sunshine.

14. At all localities the water was found to be more than four-fifths saturated with oxygen. Occasional instances of saturation or supersaturation, as the immediate effect of rapid photosynthesis, were encountered.

15. The Sörensen values were relatively high, and varied within narrow limits. The minimum value encountered was pH 7.50, at Crockett, the maximum, pH 8.16, at the Oakland Mole, thus giving a range of only 0.66 Sörensen unit for the entire bay.

16. The values for hydrogen sulfide were usually small, the maximum value encountered being 0.42 cc. per liter, at Crockett. The average for the entire bay was 0.13 cc. per liter.

17. As an index to the effect of sewage contamination, several water samples were taken at varying distances from the Channel Street sewer outlet, San Francisco. The results are shown in table 12.

18. So far as our investigations show, the chemical effect of the sewage at present discharged into San Francisco Bay is inconsiderable and is quickly dissipated by tides and currents. The most objectionable feature, biologically, of sewage contamination in the bay appears to be the accumulation of sludge on the bottom near the sewer outlets.

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EXPLANATION OF CHARTS

The times of high and low waters and of slack waters as given on the charts are those predicted for the station in question. The times of slack waters are indicated by short vertical lines between "Flood" and "Ebb." The heights of the tides given are those predicted for Fort Point. The *observed* daily ranges from lower low to higher high water at the Presidio are given for each chart.

Breaks in the graphs indicate omission of one or more samples.

CHART 1

Graphs showing results of analyses of water samples taken at Crockett, from the dock of the California and Hawaiian Sugar Refining Corporation.

The *observed* daily tidal ranges at the Presidio were: July 14-15, 6.6 feet; July 20-21, 4.5 feet; July 27-28, 7.2 feet.

CHART 2

Graphs showing results of analyses of water samples taken at Dumbarton, from the Southern Pacific drawbridge.

The *observed* daily tidal ranges at the Presidio were: July 19-20, 4.7 feet; July 26-27, 7.4 feet.

CHART 3

Graphs showing results of analyses of water samples taken at the Oakland Southern Pacific Mole.

The *observed* daily tidal ranges at the Presidio were: July 10-11, 5.9 feet; July 16-17, 6.0 feet; July 23-24, 6.7 feet.

CHART 4

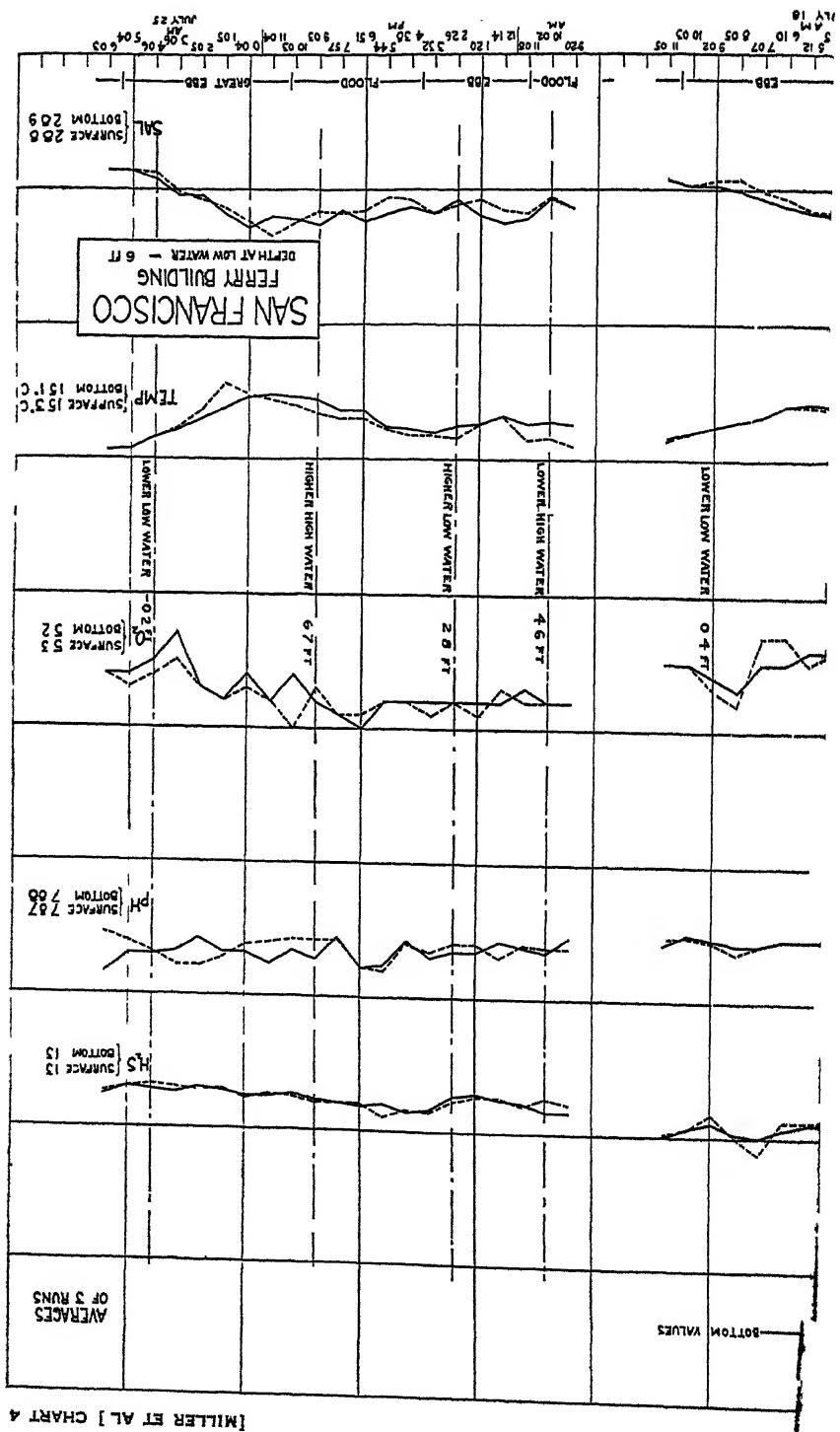
Graphs showing results of analyses of water samples taken at the San Francisco Ferry Building.

The *observed* daily tidal ranges at the Presidio were: July 11-12, 6.3 feet; July 17-18, 5.3 feet; July 24-25, 7.2 feet.

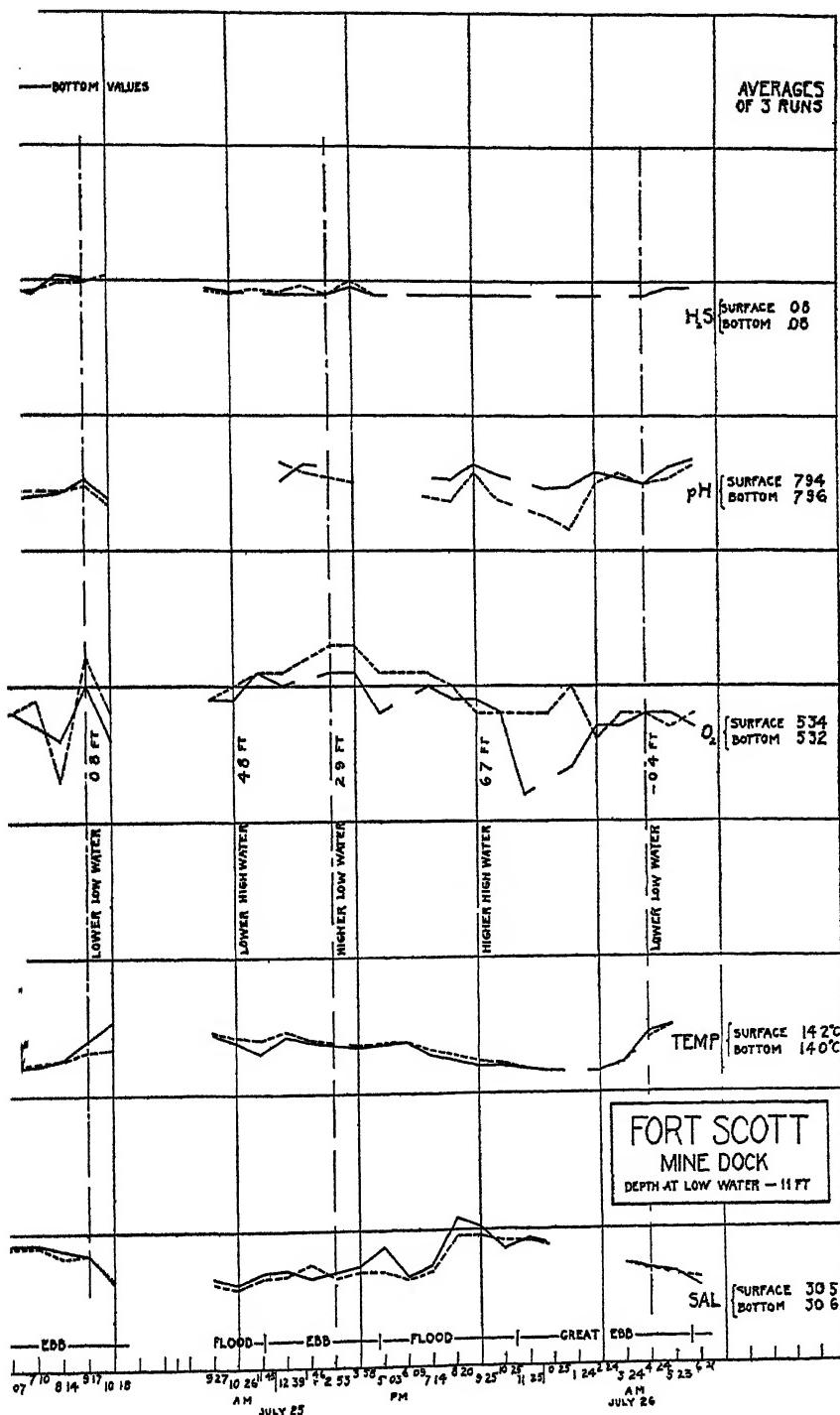
CHART 5

Graphs showing results of analyses of water samples taken at Fort Point, from the Fort Scott Mine Dock.

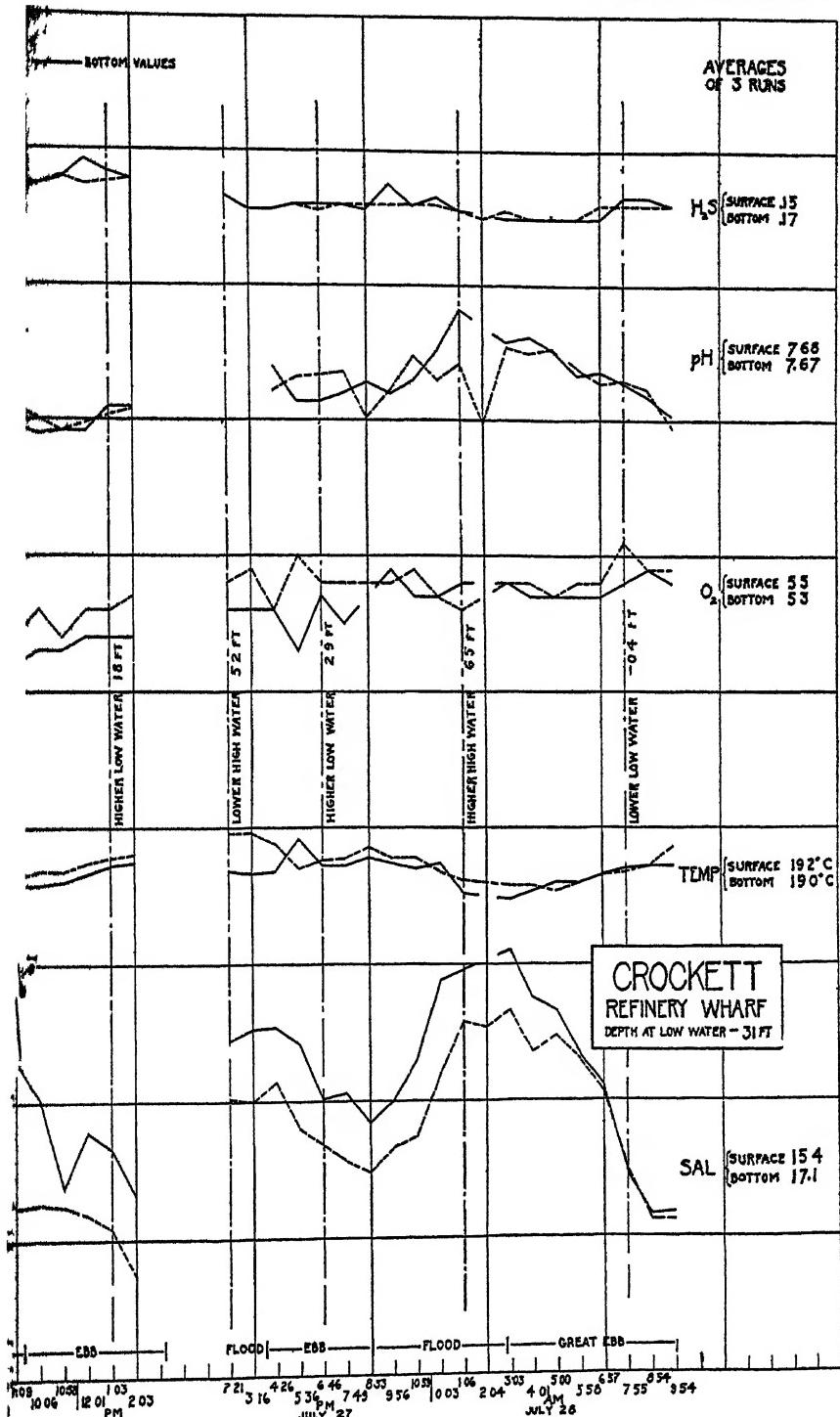
The *observed* daily tidal ranges at the Presidio were: July 12-13, 6.7 feet; July 18-19, 5.0 feet; July 25-26, 7.3 feet.



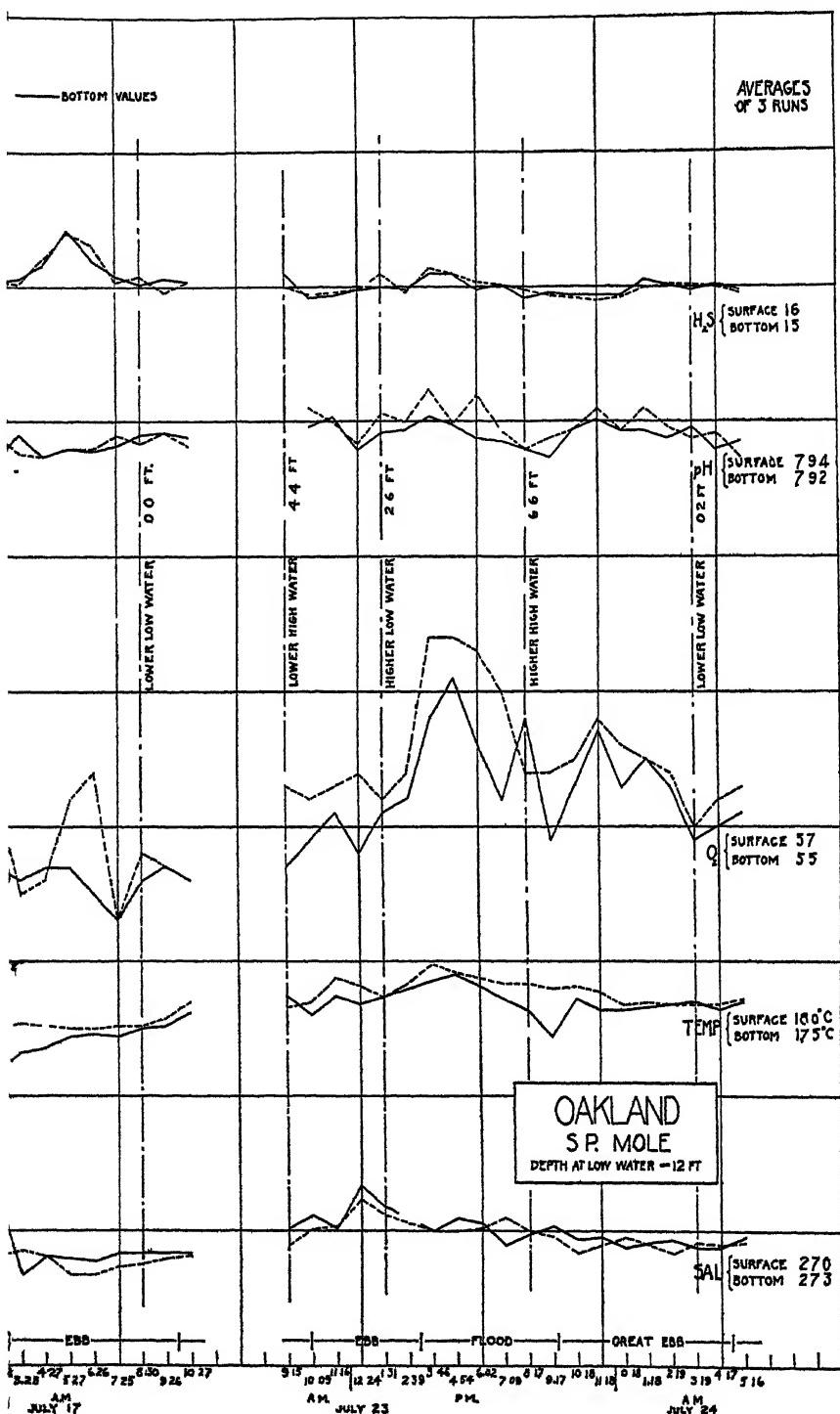
MILLER ET AL / CHART 4

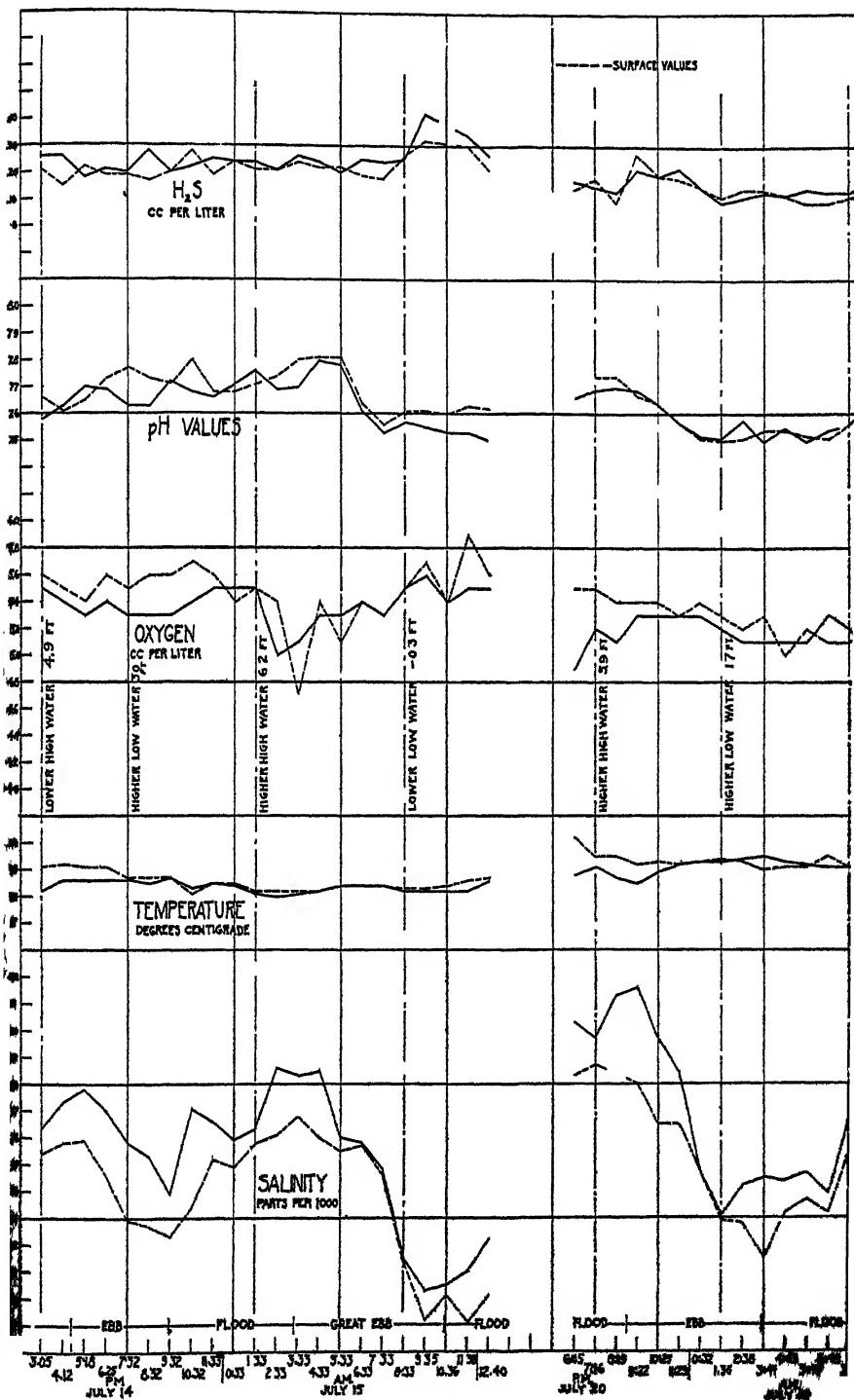


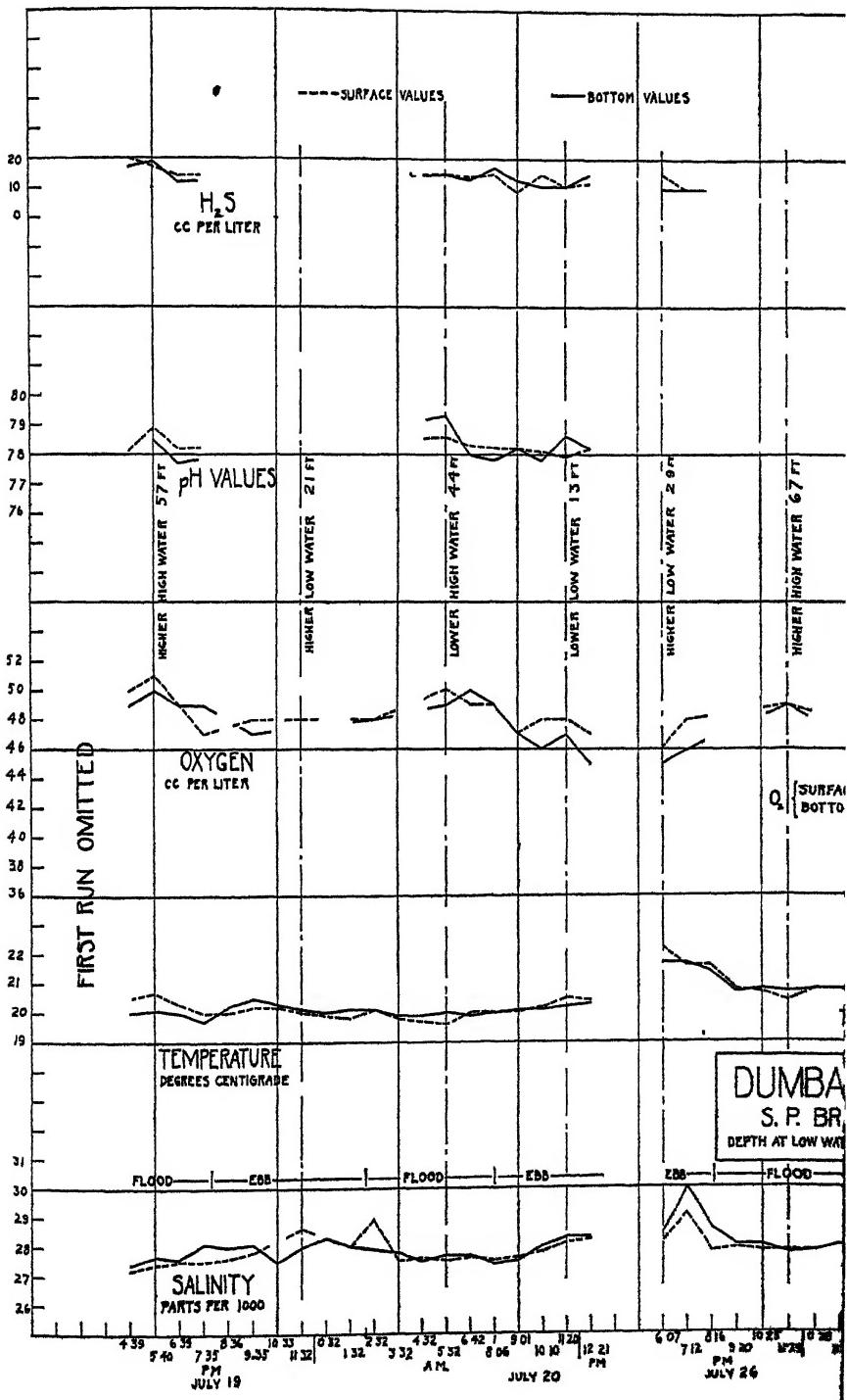
[MILLER ET AL.] CHART 1

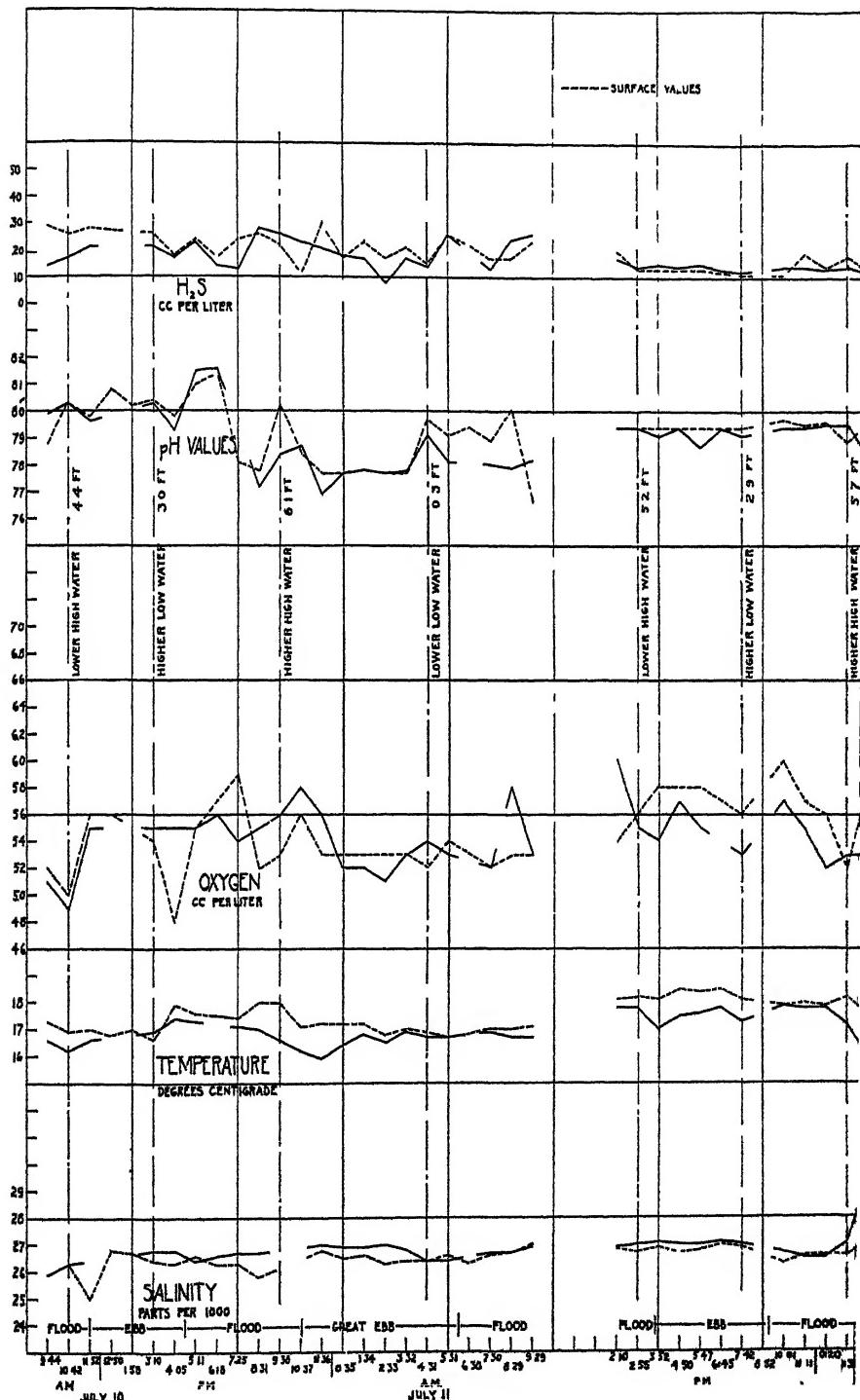


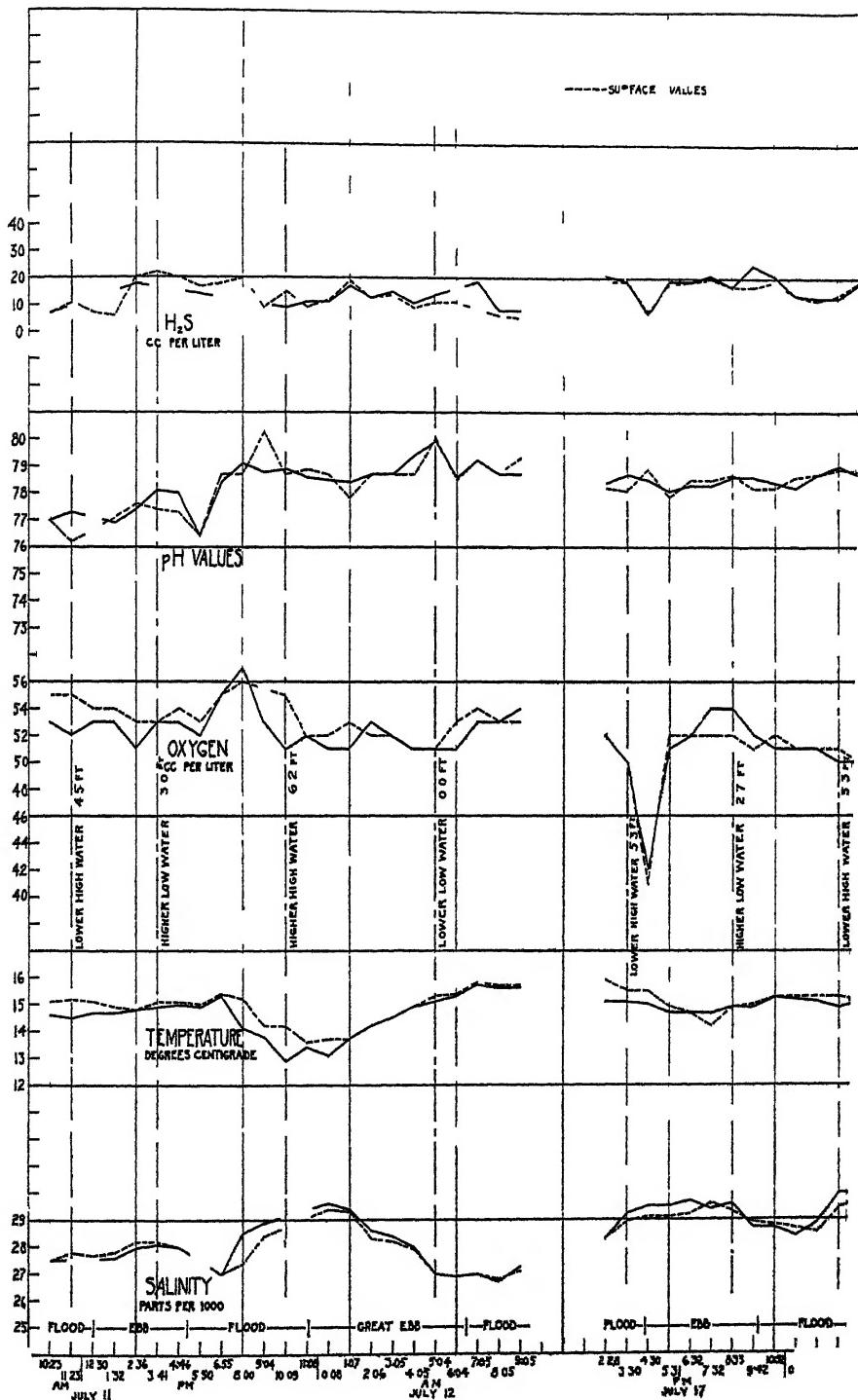
[MILLER ET AL.] CHART 3

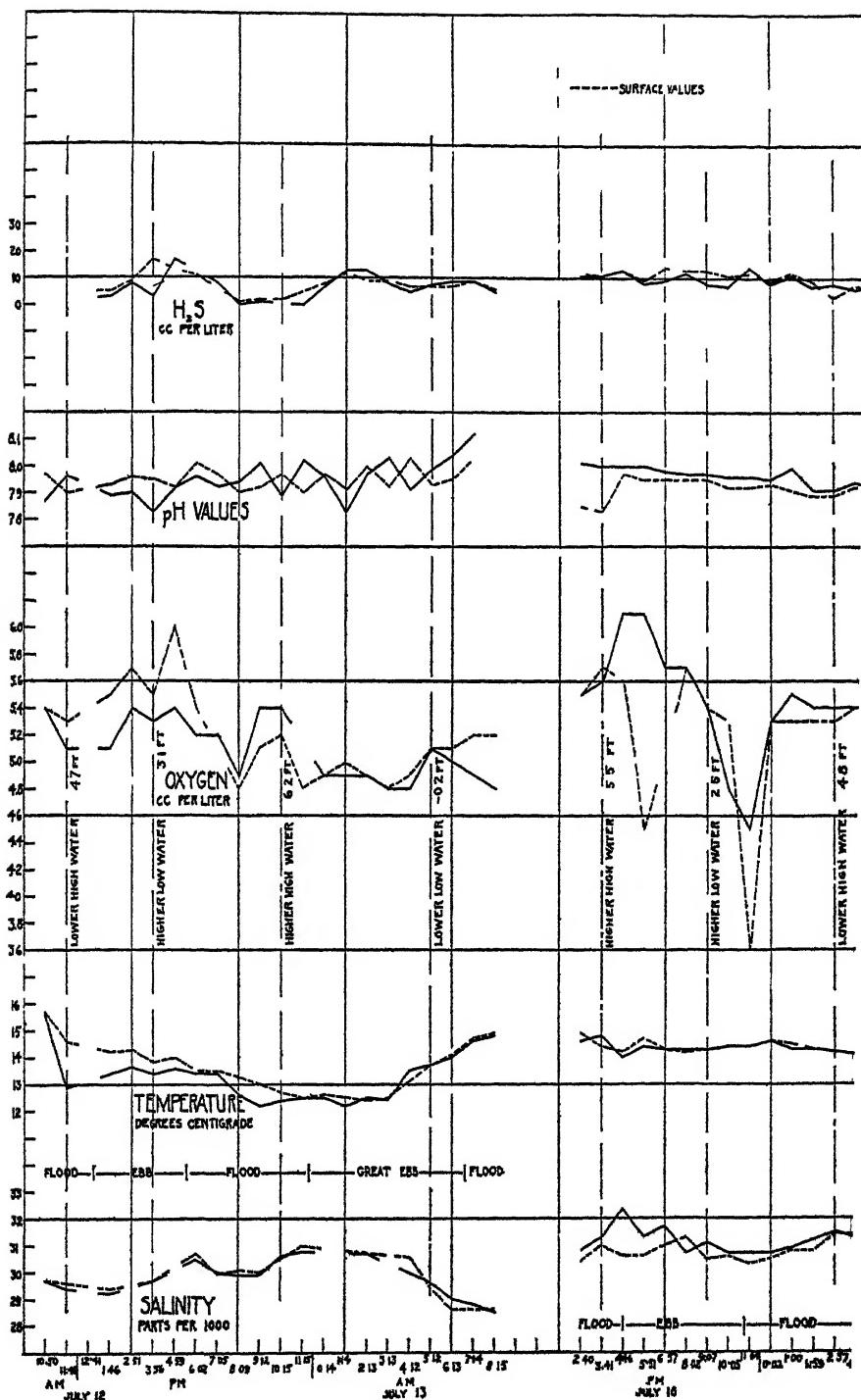












EXPERIMENTAL TRANSFAUNATION OF
TERMITES

BY

S. F. LIGHT AND MARY F. SANFORD

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 12, pp. 269-274, 2 figures in text

Issued July 18, 1928

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

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The amazing protozoan faunas which inhabit the hind-gut of the termites of the lower families have long been known. Increasing knowledge shows the faunas of the different termite species to be characteristic, each faunate member of every colony of a given species examined throughout its range having in its hind-gut the same species of Protozoa, some or all of which are found nowhere else.

The question immediately presents itself. Is this constant specific association due to an obligatory host-guest relationship or to the isolated habit of life of termites? Since Cleveland (1924) has shown the termites to be dependent upon the Protozoa for their ability to use cellulose as a food, the problem presented is really dual, (1) Can the Protozoa of one termite species live and multiply within the hind-gut of another termite species?, and (2) Is the termite host able to adjust itself to the foreign protozoan fauna and function normally therewith? There arise, also, the questions of the possibility of mixed faunae and the differential survival abilities of the different species of Protozoa concerned in various termite hosts.

The present note presents experimental results which show that the protozoan fauna of at least one species of termite can live for considerable periods of time, at least several months, within the intestine of a widely different species of termite. These results seem to indicate also that the Protozoa multiply within the new host, although further experiment is needed definitely to prove this point. A similar result was indicated by the preliminary experiments reported by us (1927) in which Protozoa from *Termopsis* lived for some days within the gut of a defaunate *Porotermes* nymph.

The results given here concerning the ability of Protozoa of *Termopsis* to live and multiply within the hind-gut of *Kalotermes hubbardi* would seem to answer the first question conclusively. It remains to be seen whether this ability is common to the Protozoa of the termites or peculiar to those of *Termopsis*. It must be pointed

out that such desultory attempts as have been made to transfaunate *Termopsis* with faunas of other species have so far proved unsuccessful. These negative results are far from conclusive, however, and may well be due to the mechanical problems involved, (1) the difficulty of injecting *Termopsis* per anus because of the tendency of the insect to extrude violently its own intestinal contents upon the slightest stimulation and (2) the difficulty of collecting sufficient intestinal fluid when the smaller species serve as donors.

MATERIALS AND METHODS

For these experiments the local *Termopsis* (supposedly *T. angusticollis* Hagen) was used as the donor of intestinal material, with *Kalotermes hubbardi* Banks as the host. The *Termopsis* nymphs and soldiers were obtained from decaying logs in Golden Gate Park, San Francisco, save those used in the last experiment (Kh i T₄) which were from Mokelumne Hill, Calaveras County, received through the kindness of Miss Genevieve Emerson. The *Kalotermes* material was obtained from Phoenix, Arizona, where it abounds in the cottonwood trees. For this abundant and splendid material we have to thank Mr. Henri Behoteguy and Mr. Donald O. Williams, both of Phoenix.

The fauna of *Termopsis*, aside from the smaller tetramitids, consists of *Trichonympha campanula* Kofoid and Swezy, *Leidyopsis sphaerica* Kofoid and Swezy, *Trichomonas termitidis* (Kofoid and Swezy), and *Streblomastix strix* Kofoid and Swezy. That of *Kalotermes hubbardi* consists of the strikingly different forms *Staurojoenina* sp. and *Metadevescovina debilis* Light.

Defaunation was readily and completely accomplished without injury to the termites by oxygenation as first suggested by Cleveland (1925). For this purpose compressed commercial oxygen ("Purox") in tanks was used and a simple but effective oxygenation chamber devised (fig. 1). This consists of a 6-inch length of hydraulic tubing 6 inches in diameter with $\frac{1}{4}$ -inch walls and a base of $\frac{1}{4}$ -inch steel electro-welded. The lid is finely threaded and fitted with a rubber gasket. The oxygen intake is below with a valve. The outlet is above with a small pressure gauge and a stopcock.

To remove or dilute the contained air sufficiently, the oxygen was allowed to flow through the apparatus for ten minutes. Since this was at once successful no accurate information was sought as to

the minimum flow or length of time necessary to dilute the air sufficiently to make it effective for defaunation, nor was the minimum time for defaunation at given pressures tested. Such data as were obtained agreed in general with Cleveland's findings (1925). The oxygen had no apparent harmful effect upon the termites and therefore they were given long treatments in order to insure complete defaunation. All the *Kalotermes* used in the experiments here reported were left for two to two and one-half hours in oxygen at a pressure of from 40 to 50 lb., i.e., between two and three atmospheres. They were removed from wood and placed with a little moist filter paper in a closed circular cage of fine-meshed copper screening in which they were placed within the hydraulic tubing.

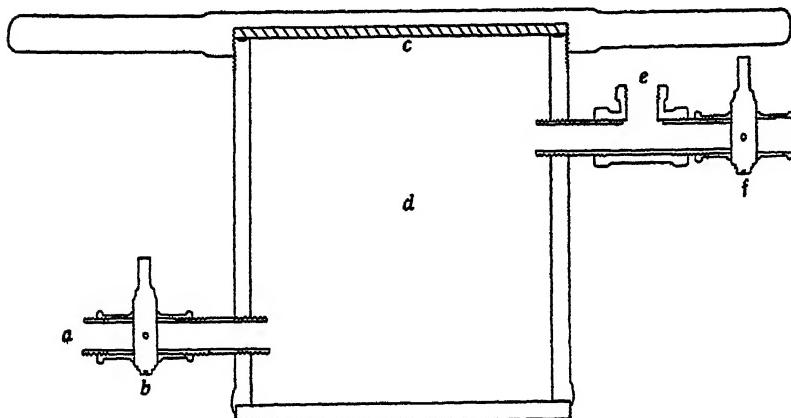


Fig. 1. Plan of oxygenation apparatus in vertical section. ca. $\times \frac{2}{3}$. *a*, inlet connected to oxygen tank; *b*, inlet valve; *c*, sheet rubber gasket in lid; *d*, chamber; *e*, T-joint for pressure gauge; *f*, outlet stopcock.

Two lots of the defaunated termites were injected immediately, the third, two days, and the fourth (*Kh i T₄*) thirteen days after defaunation. The correlation between delayed injection and viability under laboratory conditions, which will be noted, may be either entirely or in part apparent, viability being a matter dependent largely upon such factors as injury in handling, moisture supply, fungus attack, etc., or it may be that the period allowed for adjustment to the death and disintegration of the Protozoa before being injected with a foreign fauna was conducive to longer survival.

METHODS OF INJECTION

The defaunate stock and the prospective donors are placed in separate open dishes. With the pipette (fig. 2) in readiness, the head of a donor nymph is crushed and the intestinal content, given up as a result of this operation and of gentle pressure on the abdomen, is carefully sucked up into the pipette. The prospective host is quickly picked up and placed between thumb and first and second fingers of the left hand, where it is held by a firm yet gentle grasp, with the

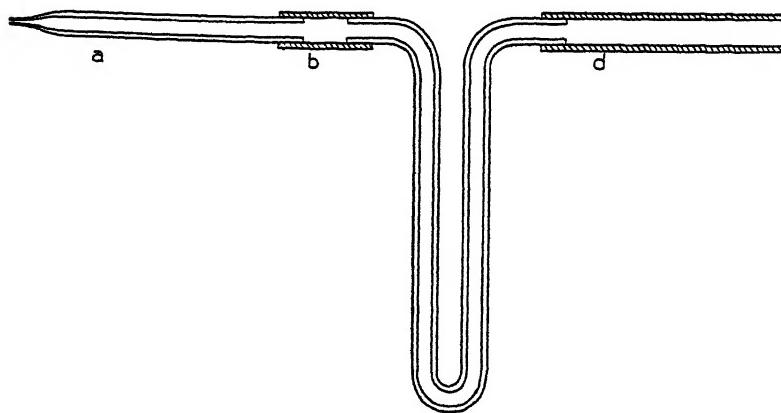


Fig. 2. Pipette used in anal injections. $\times \frac{2}{3}$. *a*, pipette; *b*, rubber tubing junction; *c*, bent glass tube which acts as a guard, preventing fluids or particles in *d* from being blown into pipette; *d*, rubber tubing of convenient length serving as a mouthpiece.

tip of the abdomen visibly protruding. The tip of the abdomen and the tip of the fluid-filled pipette are brought into view under dissecting binoculars. Gentle pressure on the abdomen or slight rotary movement of the fingers or both is generally sufficient to cause the prospective host to give up a drop of intestinal fluid. The tip of the pipette is then gently inserted into the anal aperture and the contents slowly forced into the hind-gut by blowing gently but steadily into the rubber tubing attached to the pipette. The pipette is slowly withdrawn and the transfaunated individual placed in the appropriate receptacle.

Speed is the important factor in the whole operation in order to avoid the drying of the Protozoa and, even more important, their

deterioration because of exposure to atmospheric oxygen. There are indications that injections repeated at an interval of a day or two may succeed when single injections fail.

Kalotermes hubbardi used as host is not a favorable laboratory animal. The need for a considerable amount of moisture on the one hand and the danger from fungus attack on the other, sets a rather low life-limit for a small group of individuals in the laboratory. The period of life of the four injected groups is not unlike that observed for perfectly normal faunate or defaunated groups under similar conditions. The question of the termites' ability to function normally with a foreign fauna was not under consideration, however, and hence no steps were taken to insure perpetuation of the colonies. This and other connected questions are under investigation at the present time.

EXPERIMENTS

Number	Donor	Host	Date of defaunation	Date of injection	Last date of finding normal fauna	Number of days living in foreign gut
Kh i T ₁	<i>Termopsis</i>	<i>Kalotermes hubbardi</i>	Nov. 9, 1927	Nov. 9, 1927	Jan. 10, 1928	61
Kh i T ₂	<i>Termopsis</i>	<i>Kalotermes hubbardi</i>	Nov. 9, 1927	Nov. 11, 1927	Feb. 8, 1928	90
Kh i T ₃	<i>Termopsis</i>	<i>Kalotermes hubbardi</i>	Nov. 16, 1927	Nov. 16, 1927	Jan. 11, 1928	56
Kh i T ₄	<i>Termopsis</i>	<i>Kalotermes hubbardi</i>	Nov. 16, 1927	Nov. 29, 1927	Mar. 8, 1928	100

The purpose of these experiments was to determine the possibility of continued existence of *Termopsis* Protozoa in the *Kalotermes* gut and also, if possible, whether or not they were able to reproduce in the new environment. Effort was therefore made to keep the groups of transfaunated individuals alive as long as possible. Examinations were made only on single individuals and, until the end, usually only on moribund specimens or on those which exhibited signs of being abnormal. Since similar moribund individuals from a normally faunate colony often show reduction of fauna or the entire loss of certain species or even of the entire fauna, certain negative findings are not necessarily any index of the ability or lack of ability of the Protozoa to live in the new environment but rather of the health of the individual termite concerned, and are of no significance here. In reporting the experiments, therefore, only the last date at which a

normal fauna was found in a transfaunated individual and the period of time during which the Protozoa lived in the new environment are given. Since in all cases the last active individual remaining alive showed a protozoan fauna in apparently healthy condition, the period during which the Protozoa lived, had apparently nothing to do with the ability of the Protozoa to live in normal conditions in the host termites but was limited by the conditions which caused the death of the termites under laboratory conditions. Since defaunate *Kalotermes* lived 71 days under similar conditions and since faunate *Kalotermes* seldom live for a markedly longer period under the same laboratory conditions than did those transfaunated, there seems no reason to believe that the length of this period was limited by the presence of foreign Protozoa. The experiments simply show the ability of the Protozoa of *Termopsis* to live within the gut of *Kalotermes* for at least 100 days, and to multiply there. This would seem to disprove any obligatory biochemical relation of the Protozoa of *Termopsis* to the conditions in the *Termopsis* gut. Whether the new host is able to continue to function properly with its foreign fauna remains yet to be proved.

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INTESTINAL PROTOZOA OF MONKEYS

BY

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UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 13, pp. 275-306, plates 12, 13

Issued August 11, 1928

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

INTESTINAL PROTOZOA OF MONKEYS*

BY

JOHN F. KESSEL

INTRODUCTION

References to intestinal Protozoa of monkeys which are similar in appearance to the intestinal Protozoa of man have appeared from time to time in the literature. One group of investigators has preferred to regard as a new species each Protozoan found in a species of primate not previously recorded as harboring that parasite, while the other group tends to regard such Protozoa as probably identical with intestinal Protozoa found in man. Dobell (1919) and Dobell and Laidlaw (1926), though apparently somewhat in doubt on the subject, incline to the latter view, while Wenyon (1926) is noncommittal, although he accepts the names *Endamoeba pitheci* Prowazek 1912 and *Endamoeba nuttali* Castellani 1908 as being the correct names for the amoebae in monkeys, resembling *E. coli* and *E. dysenteriae* respectively. On the basis of host environment exclusively, Brug (1923) concludes that the amoebae of monkeys are of different species from those found in man.

Since most of the investigators hold that the intestinal Protozoa of monkeys are morphologically indistinguishable from the Protozoa of man, the question of their species identity or difference must be settled on physiological rather than on morphological grounds. Physiological characters may be determined from

- a. the results of cross animal infection,
- b. the growth and reaction of the Protozoa in culture media,
- c. the pathological effects on a standardized experimental animal such as the kitten.

The present report is concerned with both the morphological and physiological characteristics of the intestinal Protozoa of monkeys.

The following tabulation gives the more important references to intestinal Protozoa of monkeys:

* Contribution No. 91 from the Parasitology Laboratory, Department of Pathology, Peking Union Medical College.

AMOEBAE

ENDAMOEBA DYSENTERIAE

Author and date	Species of monkey and locality	Species of Protozoa	Remarks
Musgrave and Clegg (1904)	<i>Macacus cynomologus, M. philippinensis.</i> Philippine Islands.	<i>E. dysenteriae.</i>	Naturally contracted amoebiasis, with ulceration of colon.
Castellani (1908)	<i>M. pileatus.</i> Colombo.	<i>E. nuttali.</i>	No lesions in the bowel but spontaneous amoebic liver abscess.
Noc (1909)	Saigon.		Cysts 10-12 μ in diameter; monkeys suffered from spontaneous dysentery.
Prowazek (1912)	Orang-outang. <i>Simia satyrus.</i>		Plate 18, figure 27, shows cyst of <i>E. dysenteriae</i> ; monkey suffered from colitis, showing blood and mucus in stools.
Kartulis and Strong (1913)	Orang-outang. Manila.		Appendicitis and amoebic liver abscess.
Mathis (1913)	<i>M. rhesus,</i> <i>M. tcheliensis.</i> Tonkin.	<i>Löschia duboscqi.</i>	
Swellengrebel (1914)	<i>M. rhesus.</i> East Indies.	<i>E. chattoni.</i>	<i>E. histolytica</i> type.
Behrend (1914)	<i>M. rhesus.</i>		Cysts of both <i>E. dysenteriae</i> and <i>E. coli</i> .
Macfie (1915)	<i>Cercopithecus petaurista.</i> West Africa.	<i>E. cercopitheci.</i>	Associated with dysentery which Macfie judged to be caused by <i>E. dysenteriae</i> .
Eichhorn Gallagher (1916)	<i>Ateles ater</i> South America	<i>Ameba ateles.</i>	Severe outbreak of dysentery in captive monkeys; negative attempts in cats.
Dobell (1919)	<i>M. rhesus.</i>		Indistinguishable from <i>E. dysenteriae</i> .
McCarrison (1919)	<i>Macacus sinicus.</i> India.	<i>E. histolytica.</i>	Monkeys in diet experiment often developed amoebic dysentery, the amoebae present showing ingested red blood corpuscles.
Bach (1923)	16 years, <i>M. rhesus.</i> Germany.	<i>E. duboscqi.</i>	Cysts and trophozoites of amoebae resembling <i>E. dysenteriae</i> .
Brug (1923)	<i>M. cynomolgus.</i>	<i>E. cynomolgi.</i>	No symptoms.

Author and date	Species of monkey and locality	Species of Protozoa	Remarks
Fox (1923)	<i>Ateles ater.</i>	No name given but thinks the amoeba was not <i>E. histolytica</i> nor <i>E. coli</i> .	Dysentery in six captive monkeys and gross pathological findings typical of amoebiasis.
Mello (1923)	<i>Macacus.</i> Italy.	Species identical with <i>E. dysenteriae</i> .	Harbor <i>E. dysenteriae</i> often associated with dysentery; produced dysentery in kittens infected with <i>E. dysenteriae</i> of monkey.
Suldey (1924)	Chimpanzee, <i>Troglodytes niger.</i>	<i>Entamoeba atelles.</i>	Dysentery in chimpanzee; amoebae morphologically similar to <i>E. dysenteriae</i> of man and contained red blood cells.
Dobell and Laidlaw (1926)	<i>Macacus.</i>	" <i>Entamoeba histolytica</i> ."	Amoeba cultured <i>in vitro</i> and kittens infected.
Knowles, R. (1926)	<i>Macacus rhesus.</i> Calcutta.	<i>E. nuttali.</i>	Spontaneous dysentery in monkey. <i>Endamoebae</i> grew in culture and were seen to ingest red blood corpuscles.

ENDAMOEBA COLI

Brumpt (1909)	<i>Macacus sinicus.</i>	<i>Endamoeba coli.</i>	Cysts and free amoebae found.
Wenyon (1909)	Khartoum.		Cysts indistinguishable from <i>E. coli</i> .
Prowazek (1912)	Orang-outang, <i>Simia satyrus.</i>	<i>E. pitheci.</i>	Plate 18, figure 28, shows a 7-nucleated cyst which appears to be a typical cyst of <i>E. coli</i> .
Mathis (1913)	<i>M. rhesus,</i> <i>M. tcheliensis.</i>	<i>L. legeri.</i>	Attempts to differentiate from <i>E. coli</i> on morphological grounds.
Behrend (1914)	<i>M. rhesus.</i>		Cysts of both <i>E. dysenteriae</i> and <i>E. coli</i> figured.
Mello (1923)	<i>Macacus.</i>	<i>E. coli.</i>	Also describes <i>Amoeba multinucleata</i> , which is probably <i>E. coli</i> .
Dobell and Laidlaw (1926)	<i>M. rhesus.</i>	" <i>Entamoeba coli</i> ."	Unable to distinguish morphologically from <i>E. coli</i> , cultured successfully <i>in vitro</i> .

ENDOLIMAX NANA

Brug (1923)	<i>M. cynomolgus.</i> Java.	<i>Endolimax cynomolgi.</i>	Resembles <i>E. nana</i> of man.
Dobell and Laidlaw (1926)	<i>Macacus.</i>	<i>Endolimax nana.</i>	Concludes that it is the same species as <i>E. nana</i> of man; have cultured successfully.

IODAMOEBA BÜTSCHLII

Author and date	Species of monkey and locality	Species of Protozoa	Remarks
Brug (1920)	<i>M. cynomolgus.</i> Java.	<i>Endolimax kueneni.</i>	First described as differing from <i>Iodamoeba</i> of man because of darkly staining area, later (1923) this idea retracted though species name was retained.
Hegner and Taliaferro (1924)	<i>Cebus variegatus.</i> Brazil.		
Dobell and Laidlaw (1926)	<i>Macacus.</i>	<i>Iodamoeba bütschlii.</i>	Cultured successfully.
Wenyon (1926)	Gorilla.		

FLAGELLATES

GIARDIA

Fonseca (1916)	<i>Cebus caraya.</i> South America.		Regarded as identical with <i>Giardia</i> of man.
Hegner (1924)	<i>Atelus geoffroyi.</i>		Inclines to view, on basis of size, that species is different from that found in man.

CHILOMASTIX

Prowazek (1916)	<i>Simia satyrus.</i>	No name.	No description.
Bach (1923)	<i>M. rhesus.</i>	No name.	No description.
Hegner (1924)	<i>Cebus apella.</i>	No name but inclines to view that species is different on basis of size of cysts.	Cysts are similar in shape though said to be larger than cysts from man.

TRICHOMONAS

Brumpt (1909)	<i>M. sinicus.</i>
Prowazek (1912)	<i>Simia satyrus.</i>
Mello (1923)	Orang-outang.

EMBADOMONAS

Fonseca (1917)	<i>Cebus carya.</i> Brazil.	<i>E. wenyonii.</i>	Wenyon (1926) says the division stages of this form are similar to those of <i>E. intestinalis</i> .
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TRICERCOMONAS

Dobell and Laidlaw (1926)

CILIATES

BALANTIDIUM COLI

Brumpt (1909)	<i>M. cynomolgus.</i>	<i>B. coli</i> of monkey transferred to pig.
Walker (1913)	Philippine Islands.	Monkeys infected with <i>B. coli</i> of pig and man.
Hegner and Holmes (1923)	<i>Cebus variegatus.</i>	Variation in measurements.

MATERIALS AND METHODS

The investigations here reported extended from January, 1924, to January, 1927, and were made upon eight monkeys used by various departments of the Peking Union Medical College and upon twelve others purchased especially for this study in local markets in Peking, for the most part imported from the south of Asia. Four species were used, all belonging to the genus *Macacus*. Unfortunately a number of the monkeys died soon after their purchase and it was impossible to secure more than a brief record of their intestinal fauna and observations on the condition of the intestinal tract at autopsy. Nothing is known of the history of these monkeys prior to their purchase, a point that must be borne in mind with reference to this investigation as well as with reference to all other observations on monkeys in captivity. While one may be dealing with naturally acquired infections of Protozoa, there is no positive assurance that the infections were acquired in the natural state before captivity, there being always the possibility that the infections were of human origin.

Routine examination of the monkey feces was made in the same manner as with human feces in the Parasitological Laboratory of the Peking Union Medical College (Kessel, 1924). Donaldson's iodine-eosin stain was used for preliminary examinations and smears stained with Haidenhain's iron-haematoxylin were kept as permanent records. Except where otherwise stated the intestine was fixed either in Zenker's or Schaudinn's fluid within a few minutes after the death of the animal. This was followed by sectioning and staining, usually with haematoxylin-eosin.

The details of the experimental work are given in the body of the paper.

PROTOZOA

Intestinal Protozoa encountered in monkeys prior to their being used for any experimental work are shown in table 1. This is a much higher incidence than has heretofore been recorded for man. Since the evidence derived from this investigation favors the conclusion that the intestinal Protozoa of monkeys observed in this study are identical with the intestinal Protozoa of man, the names used in identifying human Protozoa will be employed in this discussion.

TABLE 1
ORIGINAL PROTOZOAN INFECTIONS OF MONKEYS

		Monkey No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
		Species	M I	M I	M I	M S - J	M L	M I	M I	M S - J	M R	M R	M R	M R	M R	M R	M R	M R	M L	M L	M L	
PROTOZOA																						
<i>E. dysenteriae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>R. coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>E. nana</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Cryptosporidium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>I. butschlii</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Gardia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Trichomonas</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Chilomastix</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Bodo</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Endamoebas</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

M. S - J — *Macacus sinicus-johannae*
M. L — *Macacus larvatus*
M. R — *Macacus rhesus*

M. S - J — *Macacus sinicus-johannae*
M. L — *Macacus larvatus*

AMOEBAE

1. *Endamoeba dysenteriae* Councilman and Lafleur, 1891

Entamoeba nuttali Castellani, 1908.

Loschia duboscqi Mathis, 1913.

Entamoeba chattani, Swellengrebel, 1914.

Entamoeba cercopitheci Macfie, 1918.

Entamoeba cynomolgi Brug, 1923.

Entamoeba atelles Eichorn and Gallagher, 1916.

Amoebae of this species were found in eighteen of the twenty monkeys examined. The trophozoites as well as the cysts show no morphological characteristics which distinguish them from *Endamoeba dysenteriae* of man. A mensurative study of the cysts to compare the size-races with those of man has not yet been undertaken, but, as shown in plate 12, figures 13, 14, and 15, large, medium, and small size-races comparable with the size-races of the human dysenteric amoeba (Dobell, 1917) have been found. They are usually found to have ingested bacteria, but as shown by Kessel (1928) this is no uncommon phenomenon for *Endamoeba dysenteriae* even prior to their being taken from the colon of man.

EFFECT ON MONKEYS

The effect of this parasite on the monkey host seems to be similar to the effect of *Endamoeba dysenteriae* in man; namely, (1) the exhibition of acute symptoms such as dysentery or liver abscess, (2) the development of chronic amoebiasis, or (3) no symptoms apparent.

Of some twenty references in the literature to this amoeba of the monkey, twelve state definitely that its presence was associated with dysentery or with amoebic liver abscess. These cases obviously belong to the first group. The other reports do not mention any symptoms and apparently the monkeys in this group correspond to the large class of human beings who are found to harbor cysts of *Endamoeba dysenteriae* but who for the time being do not suffer from pronounced dysenteric symptoms. For convenience these may be subdivided into groups two and three mentioned above.

In this investigation, no monkeys were found to be suffering from amoebic dysentery or from amoebic liver abscess. Six monkeys of this series, positive for *E. dysenteriae*, were autopsied and the intes-

tinal tract examined in a fresh condition. Four showed an apparently normal mucosa without presence of mucus. The only amoebae found in these monkeys were in the lumen and crypts and for the most part they had ingested bacteria. In monkey No. 8 the amoebae were especially numerous in the caecum. It seems probable that these four monkeys belong to the symptomless "carrier" class. It is of interest to note that although monkeys Nos. 8 and 16 were two which showed no symptoms, yet kittens infected with amoebae from these two monkeys died of dysentery. However, in two of the six monkeys, Nos. 9 and 17, definite areas overlaid with mucus were apparent. One area in the upper colon of monkey No. 17, about eight centimeters from the caecum, showed one such mucoid area two centimeters in diameter. Smears from this area showed no amoebae on the surface, but sections taken through the region showed numerous amoebae in the mucosa and submucosa (pl. 13, fig. 1) and also in the lymph follicles (pl. 13, fig. 2). The type of penetration of amoebae into these isolated lesions seems to differ from the usual picture found in acute amoebiasis. In acute lesions the amoebae are usually found in greater numbers and very often in lakes but in this case single amoebae were found widely separated from one another, individuals often wandering alone deep into the submucosa (pl. 13, fig. 3). The intestine showed superficial necrosis and considerable infiltration of leucocytes, though no definite ulceration was apparent. The condition found in these two monkeys seems to correspond to that found in healthy Lascars reported by Acton and Knowles (1924), and is probably of the type found in a large percentage of people who show cysts of *E. dysenteriae* in their stools but exhibit indefinite or no clinical manifestations.

EFFECT ON KITTENS

Attempts were made to infect kittens with *E. dysenteriae* from four monkeys. Prior to the experiment the kittens were tested for the presence of natural infections of intestinal Protozoa, being given a high enema on three consecutive days, using a small rubber catheter. The material collected was examined microscopically and also cultured in the egg-and-serum medium of Boeck and Drbohlav (1925). For details of this phase of the work the reader is referred to Kessel (1928), from which report it will be seen that (1) eight of fourteen, or 57 per cent of the kittens injected with amoebae from monkeys, became

infected; (2) for the most part, infected kittens developed dysenteric symptoms similar to those found in kittens infected with *Endamoeba dysenteriae* of man; (3) amoebae in infected kittens were found to ingest red blood corpuscles; (4) the lesions in infected kittens were similar to those found in kittens infected with *Endamoeba dysenteriae* of man; (5) the histological sections showed varying degrees of penetrability of the amoebae into the tissue, ranging from superficial necrosis of the mucosa to penetration into the submucosa and muscularis; and (6) a slightly longer incubation period and a longer interval between the time of infection and death were noted in the series from monkeys than in the series from man.

CULTURAL OBSERVATIONS

The first amoebae from the monkey to be successfully cultured in this investigation were from cats 118 and 120, which were autopsied February 18, 1926. The amoebae were found in great numbers in hyperemic and mucus areas of the colon. They were placed in Locke's egg-serum medium of Boeck and Drbohlav (1925) and incubated at 37° C. The first examination at the end of twenty-four hours revealed trophozoites in great numbers. Mononucleate, binucleate, and tetranucleate cysts were also seen though they were not numerous. Since no cysts had been encountered in the freshly autopsied material procured on the previous day, it was concluded that encystment had taken place *in vitro*. Following this appearance of cysts only an occasional mono- or binucleate cyst appeared in the cultures except once when the temperature of the incubator was lowered by cutting off the electric current. Upon examination of the cooled cultures no trophozoites were found, although in two of the sixteen tubes cysts were seen. The tubes containing cysts were again incubated and after four days trophozoites again appeared in the culture. Amoebae from these two cats were carried on by subculturing on alternate days through eighty-one subcultures, when they were discarded.

At first human serum was used for the preparation of the medium but later horse serum was used with equal success. The amoebae grew well in media prepared with Locke's and with Ringer's solution, although comparative tests showed slightly better growth when Ringer's solution was used. Ringer's solution (Drbohlav, 1925) was therefore adopted for the preparation of all media in this study and will be referred to as R.E.S. medium (Ringer's egg-serum).

Plain agar, starch agar, and chocolate medium as outlined by Drbohlav (1925) were also tried but with much less success than the standard R.E.S. medium. Slight growth was obtained with the plain agar to which the "liquide ovomucoide" was added and more abundant growth was obtained when starch agar was used. In these media, however, cultures were secured in only about 10 per cent of the transplants and even when successfully established ten days was the maximum viability without transplanting. By adding Ringer's solution and serum (the same as added to the egg slants) growth was more abundant both on the starch agar and the plain agar. The N.N.N. medium prepared with rabbit's blood, and its modification, the chocolate medium, did not prove successful for the culture of *Endamoeba dysenteriae* from the monkey or from man. Growth was procured in the plain Locke's solution to which inactivated serum had been added as outlined by Craig (1926), but it never produced the certain results produced by the R.E.S. medium.

One modification used in preparation of the media should be mentioned, namely, the egg slants were not autoclaved since a smoother slant was procured without this additional step and since heating in the inspissator at 70° C for thirty minutes was sufficient in most cases to secure sterile media. Since sterile apparatus and solutions were used from the beginning, there was little danger of contamination from the eggs, which had been carefully brushed with alcohol.

After learning of the experiences of Dobell and Laidlaw (1926) in cultivating *Endamoeba dysenteriae*, minute granules of rice starch have been added to the R.E.S. medium and also to the solution of Ringer's and the inactivated serum solution (Craig's medium). In the series thus far tried, the addition of the starch accelerated multiplication and growth, and greater success was assured in starting cultures by the addition of starch to the medium. There was no opportunity to attempt infection experiments with amoebae growing in media to which rice starch was added. In addition to the amoebae from the monkey derived from kittens Nos. 118 and 120, which were cultured successfully, amoebae from kittens Nos. 116, 121, 123, and 124 were similarly cultivated in the R.E.S. medium.

Amoebae obtained directly from monkeys Nos. 8 and 16 were cultured in this medium and also in the R.E.S. medium to which rice starch granules were added. These amoebae were cultivated both by planting the active trophozoites obtained from the fecal material directly into the medium and by planting fecal material which had

remained at room temperature for two days and which showed the presence of cysts only. This excystment was first noted by the writer before the work of St. John (1926), Yorke and Adams (1926), and Dobell and Laidlaw (1926) was reported and it has since been repeated with amoebae both from monkeys and from man. In order to produce maximum excystment *in vitro* the writer has found, in confirmation of Dobell and Laidlaw (1926), that the cysts should be out of the body of the host for at least a twenty-four hour period.

Dobell and Laidlaw (1926) state that they have been unable to induce *Endamoeba dysenteriae* of the monkey to ingest human red blood corpuscles *in vitro*, and these workers cite this as a possible criterion for differentiating the amoeba of man from that of the monkey. Knowles (1926), however, in referring to *Endamoeba dysenteriae* of the monkey, says, "The entamoeba grew very well in culture and was seen to ingest red blood corpuscles." Contrary to the prevailing idea that *E. dysenteriae* of man will always ingest red blood corpuscles with facility, the writer has found that well fed amoebae from culture often fail to ingest red blood corpuscles. Very often, however, the same amoebae, if changed from a richer media to a plain serum overnight, will then ingest red blood corpuscles. This is not always the case, however, and some strains from man derived from feces in which four-nucleate cysts alone were present were never seen to ingest red blood corpuscles, though repeatedly given the opportunity.

This same characteristic applies to *Endamoeba dysenteriae* of the monkey. Amoebae from monkey No. 16 and monkeys in the Zoological Gardens passed through cats Nos. 116 to 118 and 120, respectively, ingested human red blood corpuscles *in vitro* when the corpuscles were added to the R.E.S. medium. Amoebae derived directly from monkeys, however, did not ingest red blood corpuscles so readily, and amoebae obtained directly from monkey No. 16 were never seen to ingest red blood corpuscles. Amoebae from monkey No. 5, however, ingested with facility red blood corpuscles, in the plain R.E.S. medium, in serum, and in Ringer's medium, though they failed to do so in medium to which rice starch granules were added.

In this investigation, *Endamoeba dysenteriae* of the monkey has been found to ingest red blood corpuscles of kittens in which it had produced dysentery, and amoebae in the intestinal tissue of monkey No. 17 were also found to have ingested red blood corpuscles. It thus seems possible for the *Endamoeba dysenteriae* of the monkey to

ingest red blood cells of the monkey, of the cat, and of man; while both this amoeba and *Endamoeba dysenteriae* of man ingest bacteria with facility in their normal habitat in the monkey and in man. Likewise, both amoebae ingest bacteria in kittens in which they have produced dysentery and both amoebae ingest bacteria and starch granules in culture.

2. *Endamoeba coli* (Grassi, 1879) Casagrandi and Barbagado. 1859

Endamoeba pithaci Prowazek, 1912.
Endamoeba legeri Mathis, 1913.

This amoeba was found in fourteen of the twenty monkeys examined. It differs in no morphological respects, either in the encysted or the trophozoite stage, from *E. coli* of man. Several binucleate cysts with nuclei in division stages have been seen and the chromosome count seems to be six, which is the same number recorded for *E. coli* of man by Swezy (1925).

The trophozoites do not show any difference from the trophozoites of *E. coli* of man. They tend to be more sluggish in their movement than *E. dysenteriae* and show no marked hyaline difference between the ectoplasm and endoplasm in the formation of pseudopodia; the only tendency toward such appearance being a narrow, hyaline margin occasionally present when the amoebae are rounded up, and just prior to their resumption of active movement.

Although this amoeba from the monkey has been in culture in the R.E.S. medium for a week, and has been transferred through three subcultures, the growth was poor and the culture died out at the end of that time. Several such attempts to culture it have given similarly poor results. In no case has successful excystment of this amoeba *in vitro* been observed in this study, although in one instance encystment occurred on the second day, following the planting of trophozoites in culture.

3. *Iodamoeba bütschlii* (Prowazek) Dobell, 1919

Endolimax kueneni Brug, 1920.

This amoeba has been found in thirteen of the twenty monkeys examined and in no way presents morphological characteristics which differ from the same amoeba found in man. In most cases the cysts show the same irregular shape and possess the characteristic glycogen

mass, and the single nucleus shows the same massed, excentric karyosome with chromatin material occasionally in the form of a crescent at the side of the nucleus opposite the karyosome. The trophozoites exhibit hyaline pseudopodia and are sluggish in their movement, never having been seen in rapid progressive movement as have *E. dysenteriae* and *E. coli*.

Brug (1920) described this amoeba from the monkey as *Endolimax kuenenii* and then endeavored to draw a morphological distinction between this amoeba and the same form in man. This was based on the presence of a darkly staining, protoplasmic area which was supposed to be present in the amoeba from the monkey and to be absent in the amoeba from man. This view of difference in morphology has since been withdrawn (Brug, 1923) though he still retains the name *Endolimax kuenenii*.

The writer has found this darkly staining area in *Iodamoeba* from the monkey but he has found it just as often in *Iodamoeba* from man (pl. 12, fig. 19); he agrees with Brug's second view (1923), i.e., that it is not distinctive for the amoeba of the monkey.

This amoeba has been cultured in the R.E.S. medium by the writer with greater ease than *E. coli* but not so easily as *E. dysenteriae*. Trophozoites have been obtained in culture both by planting trophozoites in the culture media and by planting cysts which have later excysted. In culture this amoeba does not differ from the *Iodamoeba* of man, which has been grown by planting both trophozoites and cysts.

4. *Endolimax nana* Wenyon and O'Connor, 1917

Endolimax cynomolgi Brug, 1923.

This amoeba was found in eighteen of the twenty monkeys examined and neither the cysts nor the trophozoites differ morphologically from those of *Endolimax* found in man.

Brug (1923) has called this amoeba *Endolimax cynomolgi*, on the basis of host environment alone, but Dobell and Laidlaw (1926) state that they have no hesitation in assigning the *Endolimax* of man and of the monkey to the same species. The writer concurs in this latter view.

Endolimax nana from the monkey was successfully cultured in the R.E.S. medium, together with *E. dysenteriae* and *Trichomonas*, and in pure culture. Its cultural characteristics do not differ from *E. nana* of man. Encystment was not observed by the writer.

FLAGELLATES

The intestinal flagellates of monkeys have not received the same amount of attention that the intestinal amoebae have received. This is undoubtedly because they are less frequently found and because the question of their pathogenicity is still *sub judice*. In this investigation *Giardia*, *Chilomastix*, *Trichomonas*, *Embadomonas*, and *Bodo* were seen.

5. *Giardia lamblia* Stiles, 1915

Fonseca (1916) is the first to mention seeing this flagellate in the monkey, *Cebus caraya*, though, as pointed out by Dobell (1917), some of the figures of Franchini (1912) look very much like *Giardia*.

Fonseca (1916) regarded his *Giardia* as identical with the form found in man. Hegner (1924) reports the finding of cysts of *Giardia* in a South American monkey, *Atelus geoffroyi*. He does not designate it as a new species but inclines to the view, based on Simon's (1922) size-ratio findings as a basis of species differentiation, that the *Giardia* of the monkey may be different from that of man.

In this investigation, *Giardia* was found in two monkeys, cysts being recovered in both instances and trophozoites in one. Morphologically this form appears to show the same structure as the *Giardia* from man and in order to arrive at more definite conclusions concerning the size-ratio of the cysts and trophozoites as compared with the *Giardia* of man, a number of individuals both from man and from the monkey were measured for comparison. Table 2 gives the results of this study and, for comparison, the measurements given by Simon for his *Giardia* of man and those of Hegner given for his *Giardia* of the monkey.

In the first place, it will be seen that the trophozoites of the one case of *Giardia* of the monkey found in this study, though slightly larger than of the *Giardia* of man studied by Simon, do show about the same ratio of length to breadth, i.e., 1:75, as Simon's ratio, which is 1:74.

Six hundred cysts of human *Giardia* measured in this laboratory give a length to breadth ratio of 1:61. The cysts of *Giardia* from the two monkeys observed in this study show an average length to breadth ratio of 1:59. The cysts of the *Giardia* of the monkey observed by Hegner gave a length-breadth ratio of 1:64, which is only slightly larger than the ratio obtained from man and from the monkey by the

writer. They do differ considerably from the ratio of 1:37, given by Simon for the two hundred and fifty human *Giardia* cysts that he measured. Cysts having a length to breadth ratio of 1:41 or very nearly Simon's ratio were seen by the writer in one human case in

TABLE 2

SIZE COMPARISON OF TROPHOZOITES OF *Giardia* AND OF CYSTS FROM MAN AND FROM THE MONKEY

Origin	Number Measured	Trophozoites						Ratio of length to breadth	
		Length			Breadth				
		Lowest	Highest	Mean	Lowest	Highest	Mean		
Human (Simon)	388	9.25	20.25	13.70	5.00	10.25	7.46	1:74	
Monkey 19 (Orig.)	50	12.5	19.1	14.90	6.25	11.3	8.5	1:75	
Cysts									
Human 21429	50	8.3	12	10.81	6.8	8.6	7.6	1:41	
Human 55	50	8.2	12.6	11.35	6	9.1	7.8	1:45	
Human 9571	50	10.5	13	11.89	7	8.6	7.7	1:54	
Human 101	50	9.8	12.2	11	5.6	8	7.2	1:54	
Human 4512	100	9.4	15.8	12.6	6.2	9.2	7.9	1:59	
Human 1378	50	11.2	15	12.95	6.2	8.5	8.2	1:60	
Human 21	50	11.00	13.6	12.75	6.8	8.6	7.7	1:63	
Human 378	100	9.6	14.4	12.70	5.0	9.6	7.5	1:69	
Human 507	50	8.7	12.9	11.42	5	7.5	6.3	1:81	
Human 3587	50	8.0	14.2	11.2	5	7.5	6.1	1:84	
Hum. Total (Orig.)	600	8.0	15.8	11.87	5	9.2	7.40	1:61	
Human (Simon)	250	8	14	10.7	6	10	7.47	1:37	
Monkey 19	50	9.6	14.2	11.4	5.2	8.6	7.3	1:57	
Monkey 17	50	9.5	13.5	11.2	6.3	8.3	6.9	1:62	
Monkey Total (Original)	100	9.5	14.2	11.3	5.2	8.6	7.1	1:59	
Monkey (Hegner)	100	11.01	14.4	12.69	6.77	9.31	7.76	1:64	

the Peking Union Medical College Hospital, but they were considerably shorter than the average seen by the writer and shorter than those figured by Dobell and O'Connor (1921) and Wenyon (1926). Kofoid and Swezy (1922) find that the shape of the cysts varies considerably in different races and state that the major axis varies from 1.11 to 1.7 times the transverse in length. They do not give an average ratio of length to breadth though they state that the ellipsoidal race

is the most abundant. It seems altogether possible that a larger series of cases than that studied by Simon might have afforded a different ratio value. Accordingly until other means of differentiation are found, the writer feels obliged to concur with the opinion of Fonseca (1916) that the *Giardia* of man and of the monkey belong to the same species.

6. *Chilomastix mesnili* (Wenyon, 1910)

This Protozoan was first recorded in monkeys from *Simia satyrus* by Prowazek (1912) and again by Bach (1923) from *Macacus rhesus*. No description or figures were given until Hegner (1924) recorded a *Chilomastix* from *Cebus apella*. The writer found *Chilomastix* in seven of the twenty monkeys examined and has observed both trophozoites and cysts (pl. 12, figs. 4, 5, 6).

There are no apparent morphological differences between this flagellate and the *Chilomastix* of man. The trophozoites possess three anterior flagella, the characteristic spiral shape of the body, and similar nuclear and neuromotor structures. The cysts are characteristically pyriform and have the same internal structure as the cysts of *Chilomastix* of man. Mononucleate cysts predominate but an occasional binucleate cyst was seen.

In commenting on the cysts of *Chilomastix* which he found in the monkey, Hegner (1924) states, "They resemble cysts of *C. mesnili* from man in shape but are significantly larger. Cysts of *C. mesnili* range from 7 to 9 μ in length and from 4 to 6 μ in breadth. Those from the monkey measured 8.47 to 10.26 μ in length and from 5.92 to 7.62 μ in breadth. No peculiarities in the structure of the cysts were noted."

In order to make a uniform comparison by the same individual of the size of cysts of *Chilomastix* from man and from the monkey, five cases from each were chosen at random and measured by the writer, using the same optical instrument for both series. Table 3 gives the results of this study. It will be seen that in the two hundred and fifty cysts from man the length ranged from 7 μ to 11 μ while the breadth ranged from 5.4 μ to 8.3 μ . In the same number of cysts from monkeys the length ranged from 6.5 μ to 10.65 μ and the breadth from 5 μ to 8.15 μ . The ratio of length to breadth of the cysts from man is 1:39 and of the cysts from the monkey, 1:38. These figures show very slight variation between the size of cysts of *Chilomastix* from man and from the monkey measured in this series.

TABLE 3

SIZE COMPARISON OF *Chilomastix* CYSTS FROM MAN AND FROM THE MONKEY

Origin	Number Measured	Length			Breadth			Ratio of length to breadth
		Lowest	Highest	Mean	Lowest	Highest	Mean	
Human 4024	50	8.15	9.65	8.95	5.80	7.00	6.55	1.35
Human 2033	50	7.35	10.15	9.35	5.80	8.30	6.90	1.36
Human 11801	50	7.00	11.00	9.25	5.40	7.60	6.66	1.39
Human 9035	50	7.40	10.00	9.00	5.65	8.00	6.35	1.42
Human 16230	50	7.40	10.00	9.12	5.85	7.50	6.38	1.43
Total	250	7.00	11.00	9.12	5.40	8.30	6.55	1.39
Monkey 1	50	6.85	10.00	8.60	5.15	8.50	6.60	1.32
Monkey 4	50	6.85	9.85	8.70	5.65	7.55	6.40	1.36
Monkey 2	50	7.15	10.00	9.00	5.70	7.50	6.55	1.38
Monkey 6	50	6.75	10.65	9.00	5.25	8.15	6.40	1.41
Monkey 15	50	6.50	10.35	9.10	5.00	8.15	6.35	1.43
Total	250	6.50	10.65	8.88	5.00	8.15	6.43	1.38

Although it is conceivable that there may be a racial variation in size among the flagellates, just as there is among the endamoebae, the writer does not feel that size alone is a sufficient criterion to warrant the recognition of separate species.

Chilomastix from monkey No. 5 was grown successfully in the R.E.S. medium and excystment was noted in one instance. Attempts to infect kittens were negative.

7. *Trichomonas hominis* (Davaigne, 1860)

Trichomonas from the monkey has been reported previously by Brumpt (1909) from *Macacus sinicus*, by Prowazek (1912) from *Simia satyrus*, and by Mello (1923) from the orang-outang. In the present study *Trichomonas* was found twice, once in *Macacus rhesus* (pl. 12, fig. 8) and once in *M. lasiotis* (pl. 12, fig. 9). The form found in *M. rhesus* has four anterior flagella and in respect to morphology is the same as *T. hominis* of man. The writer has no hesitancy in assigning this to the same species as the four-flagellate *Trichomonas* of man. The form found in *M. lasiotis*, however, had three anterior flagella, was larger in size, and had a much heavier trailing posterior flagellum attached to the undulating membrane than is usual in *T. hominis*. In gross appearance it reminds one more of *T. muris*.

than of *T. hominis* though the flagella are longer and the axostyle thinner than in *T. muris*. It is a larger and, structurally, a coarser appearing flagellate than the tri-flagellate trichomonas reported from man (Faust, 1921), *Trichomonas parva* of the rat, or the tritrichomonas of the pig, which are all structurally similar. Until further evidence is obtained, however, the writer is inclined to regard these differences as being racial in character rather than specific.

The four-flagellate trichomonas from *M. rhesus* was easily cultured in the R.E.S. medium and in the medium described by Hogue (1922). In culture it retained its normal number of four flagella. Kittens were successfully infected with this *Trichomonas* (Kessel, 1926b).

8. *Embadomonas intestinalis* (Wenyon and O'Connor, 1917)

Embadomonas wenyoni Fonseca, 1917.

Fonseca (1917) reported finding *Embadomonas* in *Cebus carya* in Brazil and named the form *Embadomonas wenyoni*. The writer has not had access to this paper but Wenyon (1926) inclines to the opinion that the flagellate from man and from the monkey are morphologically alike. In this investigation *Embadomonas* was found in two monkeys, *Macacus rhesus* and *M. sancti-johannis*. No cysts were noted but the trophozoites answer to the descriptions given by Wenyon (1926) for *Embadomonas intestinalis* and correspond morphologically to those found by the writer in Peking (Kessel and Svensson, 1924).

They have been successfully cultured in the R.E.S. medium.

9. *Bodo edax* Klebs, 1892

Plate 12, figure 10

Bodo was found in the feces of monkey No. 16 from time to time and at autopsy active trophozoites were found in its caecum. Similar types of *Bodo* were found in the freshly passed stools of several patients in Peking and also in the fresh caecal material of autopsied pigs. The form corresponds to *B. edax* in size and shape rather than to *B. caudatus* and the two flagella are approximately equal in length. The mouth was often difficult to detect, especially in the more rounded forms, but the two flagella were constantly present. These observations have been confirmed by others in the Parasitological Diagnostic Laboratory and, if correct, one cannot but conclude that *Bodo* may live as a commensal in the intestinal tract of mammals as well as a free-living flagellate.

CILIATES

10. *Balantidium coli* (Malmsten. 1857)

No *Balantidium* was found in monkeys examined during this investigation but brief mention will be made of records in the literature. *Balantidium* from the monkey has been reported by Brooks (1902), Noe (1908), Brumpt (1909), and by Hegner and Holmes (1923). Brumpt (1909) succeeded in infecting young pigs with his ciliate from the monkey, and Walker (1913) infected monkeys with *Balantidium coli* from man and from pigs, producing tissue invasion. One may therefore conclude that man, monkeys, and pigs may serve as hosts of *Balantidium coli*, and that experimental transfer may be made with comparative ease.

INFECTION OF MONKEYS WITH INTESTINAL PROTOZOA OF MAN

Reports of experiments in which attempts have been made to infect monkeys with the human intestinal Protozoa are meager and insufficient. Franchini (1912) claims to have produced dysentery in a healthy monkey which had been under laboratory observation by infecting it by rectum with amoebae from a case of dysentery. Amoebae were recovered at autopsy and were also found in the tissue. However, mention is not made of fecal examination of the monkey for amoebae prior to the experiment. Walker and Sellards (1913) obtained only negative results by repeatedly feeding two monkeys with cysts of *E. dysenteriae* and by attempting to infect two other monkeys with trophozoites of *E. dysenteriae* by mouth and by rectum. They however also procured uniformly negative results with their kittens which were given *E. dysenteriae* of man. Ujihara (1914) later claims to have infected a monkey with the dysenteric amoeba of man, but, as Dobell (1917) points out, his results are questionable. Dobell (1917) records two unsuccessful attempts on his own part to infect two monkeys (*M. rhesus*) with *E. dysenteriae*.

In this investigation attempts have been made to infect six monkeys with the intestinal Protozoa of man, the results being recorded in table 5. This work was done in two series, the first with monkeys Nos. 3, 5, 6, and 7, and the second with monkeys Nos. 13 and 14. These series have already been briefly reported by Kessel (1924 and 1926), but the relevant findings will be given here.

TABLE 4
MONKEYS INFECTED WITH INTESTINAL PROTOZOA OF MAN

PROTOZOA	Monkey 3	Monkey 5	Monkey 6	Monkey 7	Monkey 13	Monkey 14	Human infections established in Monkey's	
							PBF	PI
<i>E. dysenteriae</i>	+	+	+	-	-	-	+	2
<i>E. coli</i>	-	-	-	-	-	-	+	3
<i>E. nana</i>	-	-	-	-	-	-	+	2
<i>Iodamibezi</i>	-	-	-	-	-	-	-	0
<i>Giardia</i>	-	-	-	-	-	-	+	1
<i>Trichomonas</i>	-	-	-	-	-	-	-	3
<i>Chilomastix</i>	-	-	-	-	-	-	-	-

PBF—Protozoa before feeding

PI—Protozoa fed

PAT—Protozoa after feeding

PBT—Protozoa before treatment

PAT—Protozoa after treatment

Monkeys Nos. 3 to 7 were examined three times a week from January 2 to March 8, 1924, in order to determine their natural infections with Protozoa. Monkey No. 3 was negative for flagellates, monkey No. 5 was positive for *E. dysenteriae* and *E. nana* only, monkey No. 6 was positive for *E. dysenteriae*, *E. coli*, and *Chilomastix* only, monkey No. 7 was positive for all species except *Giardia* and *Trichomonas*. The monkeys were placed in separate rooms so that there was little or no danger of accidental cross-infection. Protozoa, for which they had been negative on preliminary examinations, were given by mouth.

In all cases only a single feeding was made, consisting of 5 cc. of dilute human feces containing the Protozoa indicated in table 4. The routine follow-up examinations were made from three to six weeks after the feeding, when *E. coli*, *E. nana*, *Iodamoeba*, *Chilomastix*, and *Trichomonas* were found to have been established.

Since none of these monkeys were entirely free from Protozoa it was desired to repeat the experiment with monkeys negative for Protozoan infection. None were procurable, but four young monkeys were given yatren in an attempt to free them of their intestinal Protozoa. Apparently, excessive dosage was given at first and two of the monkeys weakened and died of pneumonia during treatment. Two monkeys, Nos. 13 and 14, survived treatment and each was examined twenty-eight times between January 28 and April 20, 1925, following the treatment. Monkey No. 13 was entirely freed of intestinal Protozoa and monkey No. 14 showed a very light infection of *Iodamoeba* only. Both monkeys were fed the intestinal Protozoa indicated in the table, monkey No. 13 being given three feedings on April 22, 23, and 25, respectively, and monkey No. 14 only one feeding on April 21. As was also the case in the first series, the human feces containing the Protozoa were diluted with water and then given by mouth, using a large pipette. Cysts first appeared in the stool of monkey No. 14 on May 2, twelve days after the first feeding, and on May 11 in the feces of monkey no. 13, twenty-one days after the feeding. Subsequent examination of the feces at intervals until July 6 showed that *E. dysenteriae*, *E. coli*, *Iodamoeba*, and *Chilomastix* had been established in monkey No. 13 and *E. dysenteriae*, *E. coli*, and *E. nana* had been established in monkey no. 14.

No dysenteric symptoms were present at any time during the two months before the monkeys were autopsied. On July 6 both monkeys were sacrificed. Cysts and trophozoites of *E. dysenteriae* were

recovered from the intestinal lumen and crypts of both monkeys (pl. 13, fig. 4) but no definite pathological lesions were found. *Chilomastix* was not found at autopsy in monkey No. 13, so apparently the infection had spontaneously disappeared.

Summarizing the results of both series of infection experiments, it will be seen that *E. dysenteriae* became established in two monkeys, *E. coli* in three, *E. nana* in two, *Iodamoeba* in three, *Trichomonas* in one. *Chilomastix* in three, while attempts to establish *Giardia* were all unsuccessful. Brug (1923) also obtained negative results in attempting to infect an adult monkey with *Giardia*. These results do not necessarily indicate that such infection is impossible and merely call for other attempts with younger animals.

DISCUSSION

As already pointed out by Kessel (1924), the differentiation of intestinal Protozoa which are morphologically similar must be made on physiological grounds, the more important of which, in the light of our present knowledge being:

- a. the results of cross animal infection,
- b. the growth and reaction of the Protozoa in culture media,
- c. the pathological effects on a standardized experimental animal such as the kitten.

In the past the question has arisen whether one is justified in naming a new species of a parasitic Protozoan solely on the basis of host environment, and three tendencies have been apparent. The first has been to name new species of intestinal amoebae purely on the basis of the species of monkey in which they were found, resulting in a great variety of names in the literature. The second tendency has been to consider a Protozoan found in monkeys to be of the same species in all monkeys but different from the Protozoan of the same structure found in man. In 1919 Dobell pointed out that *Endamoeba pitheci* Prowazek, 1912, has priority for the amoeba of monkeys resembling *E. coli* in man and that *Endamoeba nuttali* Castellani, 1908, has priority for the amoeba of monkeys resembling *E. dysenteriae* of man, providing it is proved that the amoebae of monkeys belong to different species from the amoebae of man.

The third tendency has been to consider the Protozoa of monkeys as being possibly identical with the Protozoa of man. Mello (1923),

Dobell (1925, 1926), Dobell and Laidlaw (1926), and Kessel (1924, 1926) have reported experimental work on this subject. Mello appears to conclude, on the basis of structure and of the infection of kittens with the dysentery amoeba of monkeys, that *E. dysenteriae* and *E. coli* found in monkeys and in man are identical. He did, however, describe *Endamoeba multinucleata* from the orang-outang but was not prepared to draw conclusions concerning the flagellates that he found in monkeys. From his figures it seems probable to the writer that his cysts of *E. multinucleata* are merely polynucleate cysts of *E. coli* or of *Councilmania*. An amoeba resembling *Councilmania lafleuri* was found in monkeys in the present study (Kessel, 1924) but the same is not discussed in this paper because it has not been possible to procure experimental data to report at this time.

Dobell (1919) concludes that in morphology the dysenteric amoeba and the amoeba resembling *E. coli* found in monkeys are the same as *E. dysenteriae* and *E. coli* of man respectively. In 1925 he reports successful infection of kittens with the dysenteric amoeba of monkeys but finds that the kittens show symptoms slightly different from the symptoms found in kittens infected with *Endamoeba dysenteriae* of man. Dobell (1926) and Dobell and Laidlaw (1926) further report the successful culturing of the intestinal Protozoa of monkeys in the same media in which the intestinal Protozoa of man are grown and concludes that *E. nana* of man and of the monkey are the same species. However, they are noncommittal with reference to the other intestinal Protozoa of monkeys.

Most workers seem to agree that the morphological characteristics of the intestinal Protozoa of man and of monkeys are identical. If it is possible to differentiate between them at all we must therefore resort to the physiological characteristics. In this investigation the following methods were observed:

1. *Culture in vitro*.—Most of the intestinal Protozoa of monkeys have been successfully cultured, excystment and encystment having been observed in some cases (Dobell and Laidlaw, 1926, and in this study), in the same media in which the intestinal Protozoa of man have been cultured. The fact that no critical means of differentiating the Protozoa of man and of the monkey on this basis have been developed, indicates a species identity, though such is by no means established by this method alone since the egg-serum medium originally employed by Boeck and Drbohlav (1924) has been used successfully for the growth of many of the intestinal Protozoa.

2. *Animal infection.*—In order to gain adequate knowledge with reference to the cross-infections of hosts with their respective parasites it is important that reciprocal infection experiments be attempted. Unfortunately in this investigation it has not been possible to attempt infection experiments on man with the Protozoa of monkeys. Monkeys, however, have been successfully infected with *E. dysenteriae*, *E. coli*, *Iodamoeba bütschlii*, *Endolimax nana*, *Trichomonas hominis*, and *Chilomastix mesnili* of man.

Since monkeys are so heavily infected in nature with intestinal Protozoa it has been difficult to procure protozoan-free animals for experimental work. However, every precaution was exercised in examining the feces of the animals used in experimental work over a long period of time before infection was attempted and then careful isolation of the animals was exercised during the period of the experiment. While a larger series of infection experiments would afford more conclusive evidence than the present small series affords, such was impossible for the present investigation. The series does show that transmission of the intestinal Protozoa from man to monkeys is a possibility and therefore suggests that the Protozoa of monkeys may be transferred to man.

3. *Lesions produced in kittens.*—Kittens have been used more extensively in experimental amoebiasis work than any other animal because they are more susceptible to acute infection than other animals thus far employed. The details of a series of experiments on amoebiasis in kittens are reported by Kessel (1928) where kittens were successfully infected with *Endamoeba dysenteriae* of monkeys, the kittens showing the same general symptoms of dysentery, though perhaps less severe, and the same histopathological findings as shown by kittens infected with *Endamoeba dysenteriae* of man.

Dobell (1925) suggests that kittens, infected with the amoeba of dysentery of monkeys used in his series, developed symptoms somewhat different from those shown by kittens infected with the amoeba of dysentery of man. Unfortunately he has not yet stated what these symptoms are, so the writer has no means of comparison. Assuming that the same are merely differences of degree, as was also found in two of the older kittens of this series, it seems safe to conclude that the evidence from infection of kittens with the dysenteric amoeba of the monkey points to a close relationship and probably a species identity with *Endamoeba dysenteriae* of man. At least the slight differences in degree of infection appear to be insufficient to differentiate the two species.

It is possible, with improvements in methods of serum diagnosis, such as are developed for bacteria and some of the Protozoa which inhabit the blood stream, that differences now unknown may be found to separate the amoebae of dysentery of the monkey and of man into separate species, but in the light of our present knowledge one may conclude that insufficient morphological and physiological differences exist between these two amoebae to justify their separation into different species.

The species relationship of the other intestinal Protozoa of man and of monkeys cannot be so accurately determined since they are either commensals or doubtful pathogenic organisms. However, on the basis of morphological and of cultural differences *in vitro*, there are no apparent reasons why they should be separated. Dobell and Laidlaw (1926) have already stated definitely that they consider *E. nana* of man and of the monkey to be the same species and there is no obvious reason why any of the other intestinal Protozoa should be given different species names. So until definite physiological differences are produced the writer feels it is safe to conclude that the intestinal Protozoa, found in man and found in the species of *Macacus* with which he was working, in reality belong to the same species.

SUMMARY AND CONCLUSIONS

1. Intestinal Protozoa which are morphologically indistinguishable from *Endamoeba dysenteriae*, *Endamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii*, *Councilmania lafleuri*, *Giardia lamblia*, *Trichomonas hominis*, *Chilomastix mesnili*, and *Embadomonas intestinalis* found in man were encountered in four species of monkeys belonging to the genus *Macacus*.
2. With the exception of *Giardia* and *Councilmania* these Protozoa were all successfully cultured *in vitro* in the egg-serum medium in which the morphologically similar Protozoa of man have been cultured.
3. Encystment of *E. dysenteriae*, *E. nana*, *Iodamoeba*, and of *Chilomastix*, and encystment of *E. coli* and *E. dysenteriae* of the monkey were observed in culture.
4. Monkeys were experimentally infected with *E. dysenteriae*, *E. coli*, *Iodamoeba bütschlii*, *E. nana*, *Chilomastix mesnili*, and *Trichomonas hominis* of man.

5. *Endamoeba dysenteriae* of monkeys was found to have invaded the mucosa, muscularis, submucosa, and lymphatic nodules of the intestines of monkeys.

6. Kittens were experimentally infected with *Endamoeba dysenteriae* of monkeys, both by cysts and trophozoites *per anum*, and the kittens developed symptoms similar to those developed in kittens infected with *Endamoeba dysenteriae* of man.

One may conclude therefore, since neither morphological nor physiological grounds have thus far been found upon which to differentiate the intestinal Protozoa of monkeys from those of man, that the forms found in monkeys belong to the same species as those found in man. So unless more delicate methods than those thus far employed are developed we may consider the many names already given to Protozoa of monkeys as synonyms of their corresponding types found in man.

From the observations and experimental work here recorded, one may further conclude that the monkey is a very suitable laboratory animal with which to carry on experimental amoebiasis work, since the conditions of amoebiasis infection in monkeys resemble more closely than do those in any other known animal the conditions met with in human amoebiasis.

Transmitted November 1, 1927.

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EXPLANATION OF PLATES

PLATE 12

Figs. 1-3. Trophozoites of *Giardia* from monkey No. 17. All from same microscopic field. Note the difference in shape and size.

Fig. 4. Cyst of *Giardia* from monkey No. 17.

Fig. 5. Trophozoite of *Chilomastix* from monkey.

Figs. 6, 7. Cysts of *Chilomastix* from monkey.

Fig. 8. Tetratrichomonas from monkey.

Fig. 9. Tritrichomonas from monkey.

Fig. 10. *Bodo* from monkey.

Fig. 11. *Embadomonas* from monkey.

Fig. 12. Trophozoite of *E. dysenteriae* from monkey No. 16 through cat No. 118.

Fig. 13. Cyst of large race of *E. dysenteriae* from naturally infected monkey.

Fig. 14. Cyst of medium-sized race of *E. dysenteriae* from naturally infected monkey.

Fig. 15. Cyst of small race of *E. dysenteriae* from naturally infected monkey.

Fig. 16. Cyst of *E. coli* from naturally infected monkey.

Fig. 17. Cyst of *Endolimax nana* from naturally infected monkey.

Fig. 18. Cyst of *Iodamoeba bütschlii* from naturally infected monkey.

Fig. 19. Cysts of *Iodamoeba bütschlii* from man showing darkly staining protoplasmic area.

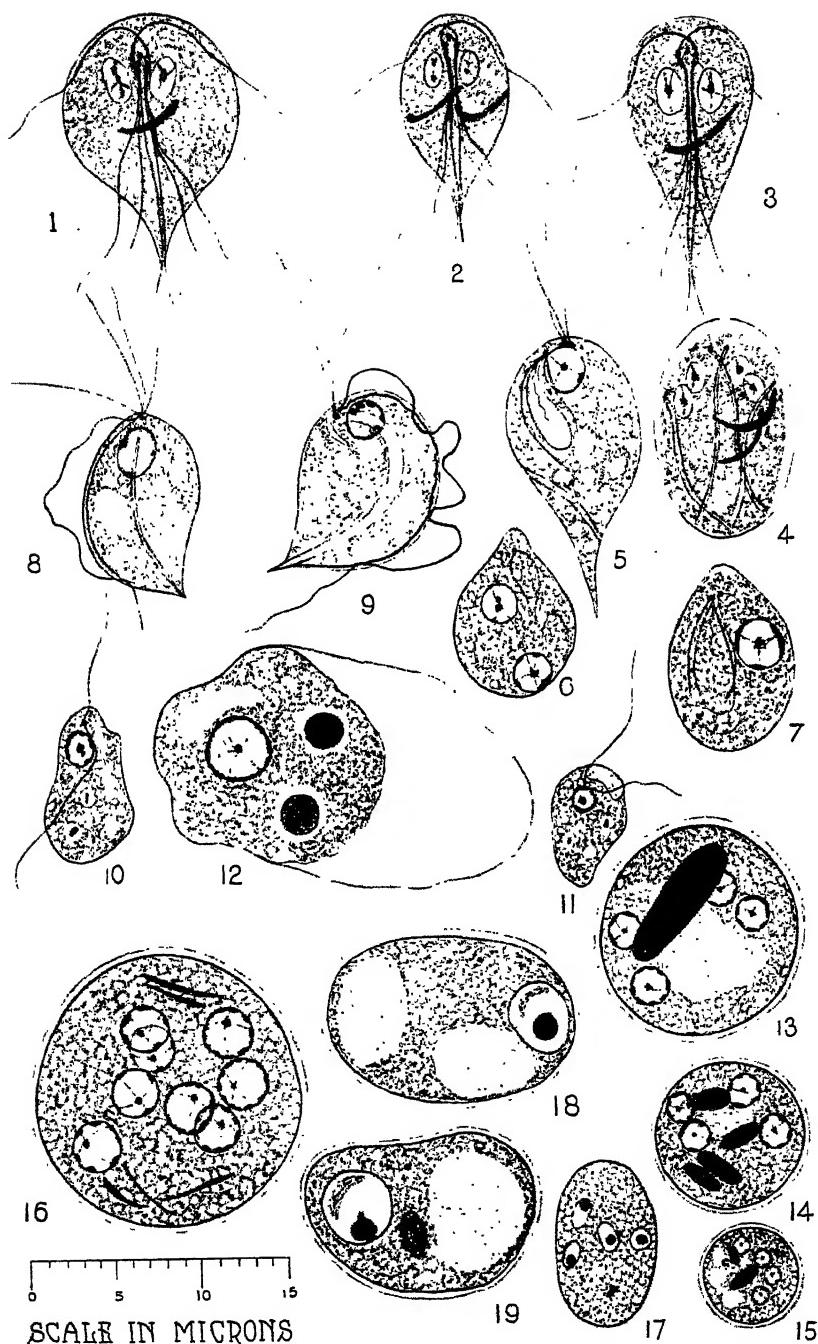


PLATE 13

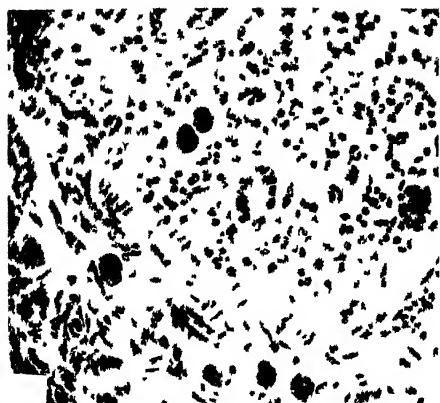
Fig. 20. Trophozoites of *E. dysenteriae* in naturally infected monkey No. 17 showing some in mucosa, some in submucosa, and some in muscularis $\times 250$.

Fig. 21. Amoebae in lymph follicle of intestine in same monkey. $\times 250$.

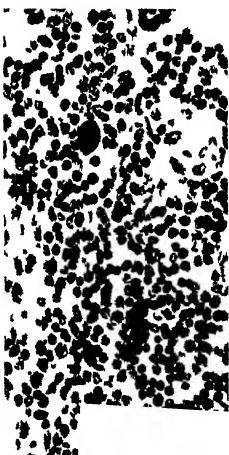
Fig. 22. Enlarged view of isolated trophozoite in muscularis of intestine of monkey No. 17. Note hyaline pseudopodium. $\times 1500$.

Fig. 23. Trophozoites of *E. dysenteriae* of man in crypts of intestine in experimentally infected monkey No. 13. $\times 200$

Fig. 24. Necrotic area in intestine of kitten No. 123 infected with *E. dysenteriae* from monkey. $\times 150$.



20



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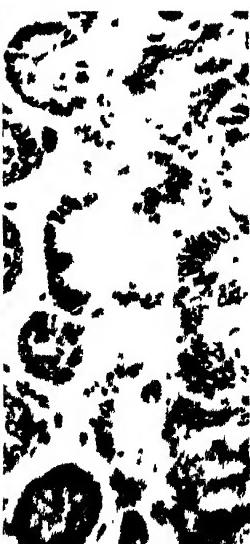
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THE ORGANISMAL CONCEPTION

ITS PLACE IN SCIENCE

AND

ITS BEARING ON PHILOSOPHY

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UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 14, pp. 307-358

Issued September 14, 1928

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

“It should be the task of the philosophical schools of this century to bring together the two streams [reflected by Kant as both scientist and philosopher] into an expression of the world-picture derived from science, and thereby end the divorce of science from the affirmation of our aesthetic and ethical experiences.”

—A. N. WHITEHEAD, *Science and the Modern World*, 218.

NOTE.—This essay should be taken as an epitome of what the senior author tried to do and did, partly, in *The Unity of the Organism* (1919); of what is contained in *The Natural History of Our Conduct* (1927); and of what is expected to be contained in the companion volume to the last now well advanced in the writing.

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THE ORGANISMAL CONCEPTION

PART I

PLACE OF THE CONCEPTION IN SCIENCE

The idea of unification and unifiedness with its concomitant idea of wholeness was never more alive and potent than it is today. So far as human society is concerned, the most explicit and influential manifestation of the conception has been the theory of society as an organism, as set forth by Herbert Spencer and others. The most definite outgrowth of the conception as applied to man is found in the extent to which present-day clinical medicine and educational theory, are recognizing the importance of "the whole man" and "the whole child."

In the natural sciences the idea has become established on numerous masses of objective reality highly diverse in character and remote from one another. The most secure bases of the idea are in those departments of natural science which deal with living beings. In attaining these solid footholds, the conception has become enmeshed in obscure details and strange nomenclature, and so has passed out of the field of vision of ordinary onlookers. Even the specialized workers often see but dimly how the happenings in their own fields conform with those in other fields.

The present writing is an effort to bring into one field of view the results of investigations in different departments of nature which justify the conclusion that, in all parts of nature and in nature itself as one gigantic whole, wholes are so related to their parts that not only does the existence of the whole depend on the orderly cooperation and interdependence of its parts, but the whole exercises a measure of determinative control over its parts.

This idea of wholeness involves the recognition that a unit exists and is possible only through the existence of parts, or elements. The conception of a unit as something uncomposed, ultimately simple, is at odds with all our best known facts. The whole is not merely something that is intact, something not torn, or cut to pieces, or smashed

into fragments. Rather a whole is something the original and necessary parts of which are so located and so functioning in relation to each other as to contribute their proper share to the structure and the functioning of the whole. A natural whole stands in such relation to its parts as to make it and its parts mutually constitutive of each other. Structurally, functionally, and generatively, they are reciprocals of one another.

Given our earth constituted as it is, and generated as it undoubtedly was, the polar zones and the equatorial zone are constitutive for each other and were generative of each other. The interrelations of these and of the whole to the totality of the bodies and conditions external to the whole earth are such that none of the zones could exist or could have come into existence without all the others and the whole. As with the earth itself, so with the conception "earth." The conceptions "earth," "polar zones," "equatorial zone," and "intermediate zones" are mutually constitutive and were mutually generative each of all the others.

This reciprocally generative and constitutive relation of a whole to its elements reaches its fullest expression in living nature. The bodies making up this portion of nature are so characteristically organized that the term organism has by almost universal consent been exclusively applied to living bodies. Vigorous and persistent analytical research in many subdivisions of living nature has shown that this peculiar relation is so dominant in living bodies as to necessitate the conceptual subordination of elements to their wholes. It is impossible to deal with the phenomena of living beings on the supposition that the elements which compose them are or can be sufficiently known to the sciences of not-living nature. The incorporation into living bodies of elements as they exist in not-living nature brings into play attributes of these elements which can be aroused in no other way. The study of living nature discovers truths about not-living nature that the study of not-living nature alone cannot discover. The organism can actualize potentialities of certain not-living substances which cannot be actualized in any other way.

The assimilation of food in our selves is a familiar manifestation of these principles. Every one of us manages, by some astounding action concerning which the most expert physiologists are almost totally ignorant, to convert the food taken into his own personal body to the requirements for his own personal activities. The word "personal" implies that what each person gets from his food is

different in some measure from what any other person gets from exactly the same kind of food. We are so familiar with these facts that their significance fails to impress us. When a competent student of the nervous mechanism tells us that "the living body shapes its own destiny, creates its own form and dynamic patterns, and organizes its own materials during and by means of its varied functions" (Herrick, 1924, p. 253); and when a competent mathematical physicist tells us that "an electron within a living body is different from an electron outside of it, by reason of the plan of the body" (Whitehead, 1925, p. 111), we begin to ask what manner of beings we are, and what is the general scheme of things of which we are parts.

As the conception of the organism as a unified whole forces its way into the biological sciences, the terms *organismal* or *organismic* gain ground. The elemental conception, in the sense that parts as we can know them as independent entities are wholly explanatory of their wholes, has proved its inadequacy in every subdivision of biology where basic problems are under investigation. This conception must be supplemented by an hypothesis which recognizes that living bodies are as real and potent in influencing the inorganic elements which they take into themselves, as these elements are in influencing the bodies which they enter. The elemental hypothesis must be supplemented by the organismal hypothesis. This does not imply the displacement of the elemental by the organismal hypothesis. It means that an adequate general concept of a living being must include the two subconcepts of a very special kind of whole and its very specially interrelated parts.

Eight sections of scientific research in which the organismal conception is showing positive and important advances have been chosen for summary examination. These sections are: cytology, protoplasmic physiology, genetical biology, biology of respiration, neural biology, endocrinology, psychology, and the sciences of inanimate nature.

CELLULAR BIOLOGY

Researches on the cell will be noticed first. Two phases of cytology have to be recognized: (1) the phase which deals with cells as elements of organisms; (2) that which deals with cells as entities in their own right, with the structures and activities within the cells themselves.

(1) *Cells as elements.*—The elementalist hypothesis has adopted a definition of "organism" which, although certainly incorrect, has found its way into nearly every elementary textbook dealing with the make-up of living bodies. The reference is to the familiar statement that an organism is an "assemblage" or "aggregation" of cells. The word aggregation means a collection. Even a student of elementary biology knows that organisms are not "built up" by any such process; any school boy can tell us that a multicellular plant or animal was at the beginning a single cell. As it increases in size, it becomes divided into a large number of cells. That the adult organism is composed of many elements called cells is a statement which instruction in elementary biology can easily verify. That these elements come into existence by a process of division within the organism as it increases in size, and are not collected together from outside sources, is a statement equally susceptible of verification.

This controversy over the question of the relation of an organism to its constituent cells has gone on from the origin of the Cell Theory to this very day. An exhaustive presentation of the controversy would have to go into the question of why the cell theory assumed to account for living beings in terms of their elements alone. Since the purpose of this article is to sketch the growth of the supplemental organismal hypothesis, we shall indicate only the salient points in the growing perception that the organism itself as a living whole is a factor in determining the nature of the cellular elements of which it is constituted.

One of the earliest expressions of the organismal conception comes from a botanist, Richard de Bary. He said: "The plant produces cells; the cells do not produce the plant." Perhaps the epigrammatic form of this statement has given it greater currency than it merits. However, it contains important truth which has become more certain with the growth of observational knowledge. Other investigators of plant development have held similar views during the entire history of the cell theory. But strongest support of the organismal conception as it relates to cells has come from students of animal development. More than thirty years ago Whitman (1893) published an essay having the title "The Inadequacy of the Cell-Theory of Development." A single sentence from this classic shows this zoologist's position relative to the organism-cells controversy:

Comparative embryology reminds us at every turn that the organism dominates cell-formation, using for the same purpose one, several, or many cells, massing

its material and directing its movements and shaping its organs, as if cells did not exist, or as if they existed only in complete subordination to its will, if I may so speak.

Such statements as this by Whitman and others of that period had less influence on biological thought than they should have had. The conception of preorganization of the egg, which these students made much use of in their efforts toward a more penetrating analysis of development, led to entanglement with the obstreperous controversy between the "epigenesists" and the "preformationists." This entanglement was sufficient to discredit much that was valuable in the work of Whitman and others of similar bent.

We close our retrospective glance at this aspect of the cell theory with a reference to the statement, published nine years ago (Ritter, 1919), that the "organism seems as much a causal explanation of the cells as the cells are a causal explanation of the organism" (vol. 1, p. 220). While this lacks definitiveness, it has been found useful to at least a few writers.

Reference to two modern writings will have to suffice to bring our review of teachings as to the organism-constituting aspect of cell life down to the present. The less recent of these is an article by the German botanist, Emil Rohde (1923). The following sentences, turned into English, present the writer's main thesis:

The "cells" of authors are not structures of morphologically equal value in the same individuality or grade or organization. They are entirely heterogeneous and of unequal value, differing fundamentally not only in their origin but in their structure and potencies.

Cells do not produce, therefore, the metaplasma but, on the contrary, cells are resultant phenomena of metaplasma-formation.

In this thesis emphasis is put on the inadequacy of the hypothesis that cells, or any of their constituents known to observational cytology, are ultimate causal units for the production or functioning of living beings. Little stress is laid on the organism as a participant in its own upbuilding and functioning. The conspicuous place assigned to the conception of plasma in the discussion gives the impression that the writer is uncertain as to the place the organism, as such, is entitled to. In other words, while this investigator is convinced of the inadequacy of the hypothesis that cells can produce organisms and make them function, he is uncertain as to what it is that cooperates with them to bring about the results we actually see.

In bringing his textbook on the cell up to date Sharp (1926) decides that it is impossible to deal satisfactorily with all the phe-

nomena in which cells are involved in multicellular organisms on the basis of the traditional conception of cells. Under the heading "The Organismal Theory" he writes:

According to this general interpretation, ontogenesis is a function primarily of the organism as a whole, and consists in the growth and progressive internal differentiation of a single protoplasmic individual, this differentiation often, but not always, involving the septation of the living mass into subordinate semi-independent parts, the cells. Since the septation is rarely complete, all parts remain in connection and the whole continues to act as a unit. (p. 73.)

The sentence which follows shows clearly that there is no disposition to deprive separate cells of any powers which can be proved to belong to them:

Thus development is not primarily the establishment of an association of multiplying elementary units to form a new whole, but rather the resolution of one whole into newly formed parts: it should be thought of not as a multiplication and coöperation of cells, but rather as a *differentiation of protoplasm*.

Cells compose the organism, as the cell theory has always contended; not, however, as the "ultimate," the "final" causes of the organism but as differentiated parts or organs by means of which the living protoplasmic mass develops and functions. These references and a mass of related evidence indicate that biologists are modifying their theories of organic elements and supplementing them by the organismal conception, so far as concerns the rôle of cells as constituents of multicellular living beings.

We now turn to the evidence that the interpretation of cell phenomena relative to the internal structure and function of cells must likewise be corrected and supplemented by the organismal conception. The examination here will be restricted to the present state of knowledge in two subdivisions of biology. One of these deals with living beings composed of what is usually regarded as a single cell. Protozoology and protophytology are the names commonly applied to this field. The other field deals with individual cells on the basis of their activities as living entities, whether as independent organisms or constituents of many-celled organisms.

So far as we are aware no protozoologist has committed himself wholly to the organismal conception. However, the swelling tide of knowledge appears to be carrying a number of them in this direction. The astonishing structural elaborateness of some of these minute creatures revealed by the methods of research now available is making a great impression on those who investigate them. That an animal

so small as to be barely visible to the unaided eye, even under the most favorable conditions, should turn out to be almost as complex anatomically as a shark, for instance, may well impress anybody, whether he has actually seen this complexity or not. That is what R. G. Sharp (1914) has shown as to *Diplodinium ecaudatum*, specimens of which can be collected from the stomach of domestic cattle. The anatomist describing this animal finds its structural parts so similar to the parts of all higher animals, that he can apply the same names used in describing the structure of the ox to this minute creature which the ox harbors in his own stomach. There are the head end, and tail end (though in this particular species no tail), and dorsal and ventral sides, of the body, the head end having a proboscis, and mouth with dorsal and ventral lips; while at the tail end there is the anus. Over the whole body is spread a cuticle, underneath which, in turn, is an ectoplasmic layer. Among the names with which published figures of the interior of the creature are labeled, we find such familiar ones as oesophagus, skeleton, contractile bundles, i.e., fiber bundles acting as muscles, and conducting fibers, i.e., fibers acting as nerves.¹

Diplodinium does not stand alone as an instance of elaborate structure among the protozoa. Professor C. A. Kofoid and his coworkers have carried particularly far in recent years the demonstration of structural complexity among them. The bringing to light in great detail of the "neuromotor" system in a whole series of species is especially noteworthy. It raises the question as to whether these creatures may be considered as fitted with the essentials of nerves and muscles, as truly as are the highest animals, and even more effectively than are some of the more lowly of the "higher ups." The conduction of stimuli by the supposedly neural part of the system of fibers has not been proved as conclusively as has that function of nerves in multicellular animals, although much indirect evidence is now at hand.²

¹ For the benefit of biologically unwary readers, it should be stated that the application of such structural terms as these in animals so widely separated in the zoological series as protozoans, sharks, and cattle, implies only that the parts named have the same function throughout the series; not that they have the same origin and composition. This difference, involving the idea of homology as distinct from that of analogy, rightly cuts a large figure in interpretative biology.

² Since this subject is one of high theoretical interest and the discoveries are too recent to have got much foothold in general zoological and neurological knowledge, the following special studies dealing with it are given in the bibliography under Taylor and Pickard: Taylor (1920); Pickard (1927).

In the light of all this, what becomes of the old notion of these minute zoons as protozoons? What becomes of their reputation as "simple minute drops of nucleated protoplasm called cells?"

These are, rather, living bodies which not only are not "built up" of cells, but which do not resolve into cells as they differentiate into organs and tissues.

There is a strong tendency among experts in this field to revert to an interpretation of protozoa presented with special fulness by the zoologist Ehrenberg. In 1838 he published an elaborate monograph having as title "The little infusion animals as complete organisms" (*Die Infusionsthierchen als vollkommene Organismen*). The investigations reported were carried out when the microscope and other aids to observation were comparatively crude. Consequently many of the "complete organisms" as seen by the investigator were considerably more imaginary than real and tended to be facsimiles on a minute scale of larger, more easily observed organisms. Time and more accurate examination have radically altered the partly real and partly imaginary descriptions he gave of them. But the rectification of his mistakes has strengthened his interpretation of the creatures as complete organisms.

Much interpretative effort in connection with microöorganisms has been directed toward proving the inadequacy of the cell theory for the task, rather than toward utilizing the organismal theory therefor. This tendency reached its peak in the reformation advocated by Dobell (1911). Dobell accepted the view, favored by many biologists, that all the "unicellular" organisms (including the bacteria as well as the protozoa) should be united under the name protista. He contended that the facts warrant regarding these as non-cellular, in contrast with multicellular organisms rightly regarded as cellular. "The concept 'cell,'" he said, "derived from a study of cellular organisms, is fairly simple. It is quite clear that the correct antithesis in the present case is between cells and not-cells, and not between many cells and one cell—as has hitherto been universally assumed." This negative idea of non-cellularity as applied to great hosts of the microscopic animal and plant worlds has not been very significant except as it reenforced the dissatisfaction previously voiced with the traditional cell theory as an aid to understanding living beings.

The following quotation from a book recently published, by a high authority (Calkins, 1926) in this field, represents the state of mind

of those protozoologists who, still having a strong attachment to the traditional cell theory, are yet conscious of insecurity in their reliance upon it for interpreting many of the facts with which they are confronted:

As organisms the Protozoa are more significant than as cells. In the same way that organisms of the metazoan grade are more and more highly specialized as we ascend the scale of animal forms, so in the Protozoa we find intracellular specializations which lead to structural complexities difficult to harmonize with the ordinary conception of cells (p. 19).

We may epitomize the present state of interpretation of the world of microscopic and ultramicroscopic living beings as follows: The incalculable myriads of organisms which for research purposes fall under bacteriology may be regarded as organisms which have never attained the full evolutional rank of cells, or which, having sometime had that rank, have lost it through degenerative evolution. Dobell's idea of non-cellularity would apply to these very well. On the other hand, all the more highly elaborated protozoa and protophyta may be regarded as organisms which have differentiated beyond the condition of true cells but without undergoing cell division as an essential step in the differentiation.

Passing to the field of biology which deals with individual cells on the basis of their activities as living entities, whether as independent organisms or constituents of many-celled organisms, we approach one of the most recondite fields of biological science, one of the latest to be entered by investigators and least familiar to people generally. The conceptions and technique of physics and chemistry applied to individual cells have yielded results which have important bearings on the organismal conception. These results are briefly summarized in the following paragraphs:

1. The most basic activities of living beings are both physical and chemical. Life phenomena are neither wholly physical nor wholly chemical. They depend on a measure of structural permanency, of constant spatial arrangements of material parts. These constitute physical phenomena. They depend also on a measure of transformation of materials, thus giving rise to new materials and energies. These constitute chemical phenomena. Living beings preserve their identity despite the basic changes which take place in them.

2. In so far as living beings are physical (as contrasted with chemical) they are "organized" in lesser or greater measure. Organization appears at the very threshold of vital phenomena as well as at

all levels of such phenomena. It manifests itself at lower as well as higher levels than cells.

3. Considerable definite information is furnished by the researches now under review as to the nature of this basic organization. Minute bodies, different from and denser than water yet suspended in water, are a *sine qua non* to any phenomena definitive of living beings. Bodies of the sort indicated maintain their identity by virtue of possessing surface films or at least layers which are separators of the bodies from, and relators of them to, their enveloping media or environments, and so are an indispensable factor to the commerce the bodies have with their environment. The most fundamental manifestations of such commerce are the responses to stimuli, resulting from the contacts between the bodies and environing bodies; and the imbibition and assimilation by the bodies of selected materials from the outside world for their own upbuilding and continued existence.

4. Investigators who study living cells with reference to their physics and chemistry designate these bodies as systems. If structure is foremost in attention "protoplasmic systems" is the term often used; while if activities are foremost, "living or irritable systems" is likely to be the term.

5. The absolute dependence of vital phenomena on the mutual relation between physical structure and chemical transformation necessitates recognition of a measure of control of each aspect of the system by the other. In such illuminating discussions in this field as those by R. S. Lillie (1923 and 1924), we find presented evidence that "living protoplasm is an example of a heterogeneous system [a cell, for example] in which the control of the chemical change by structural conditions has reached perhaps its highest development" (1923, p. 98). But we also find such statements as:

Since the energy for vital processes is in all cases transformed chemical energy, the chief effect of stimulation must be to alter the rate or character (or both) of the chemical reactions occurring in the irritable system. And since these reactions are controlled by the protoplasmic structure, stimulation must in some manner alter the structural conditions in the system. (1924, p. 187.)

That is, stimulation and conduction must "in some manner" alter the system, but without completely transforming it.

6. The conduction of stimulation from the point of application of the stimulus to other parts of the system, necessitated by the extended, or spatial, attribute of the system, is almost certainly dependent on a difference of electrical potential between the point of stimulation and

neighboring points. Thus it results that the phenomenon of conduction involves the presence of a bioelectric current, a wholly physical as contrasted with the chemical form of action.

The bearing of the results just summarized on the organismal conception is not difficult to see. The essentials of the picture before us are of bodies wholly dependent for their upbuilding and energy of action on interactions with objects of external nature. The bodies are able to preserve their identity by fixing limits to the destructive processes involved in their chemical reactions. This preservation of the bodies is due to their physical organization.

In so far as living bodies are fundamentally chemical they are elementalistic, since all chemical transformative action penetrates to the molecules, atoms, electrons, and protons of bodies. In so far as the bodies preserve their identity, that is to say, do not undergo transformative action, they are organismal. The organismal conception implies a supplementation and not a displacement of the chemically elementalistic theory of the cell.

In any adequate discussion of structure or function, surface phenomena stand out as of conspicuous importance. "Semipermeable membranes" have become as dominant from the side of physico-chemical biology as chromosomes from the side of genetical biology. The question of what such membranes are presses insistently on the mind of the general biologist. They are films or layers on the surfaces of bodies manifesting the phenomena characteristic of living beings, such bodies being entire cells or various constituents of cells, as nuclei, vacuoles, spheres, or alveoli.

Functionally the office of the membranes is to "resist the diffusion of dissolved substances." Any body manifesting vital phenomena must have certain specific materials within it which have to be obtained from the outside world, from which source the materials have to be selected, since the outside world contains many substances which would be useless or harmful were they to enter the bodies. The assertion that the partitions which separate the bodies from, but also relate them to, their outside worlds are semi-permeable means that at the same time that they function as factors in maintaining the separateness and identity of the bodies, they also allow or prevent the passage of certain materials, with regard to the best interests of the bodies at particular times and under particular circumstances.

Structurally the membranes at their simplest appear to be merely slightly condensed, perhaps slight gelations of protoplasm. In some

instances they seem to come and go depending on the conditions of the protoplasmic systems and their external surroundings. In their highest structural expression, as in the tissue cells of plants, the membranes are thick, strong, and permanent, cellulose being a common constituent of them.

Surface membranes of some degree or kind occupy a place in all processes of living beings; metabolism, nerve activity, muscle contraction, or glandular secretion. The crucial distinction between living cells and dead cells is the difference manifested by these surface layers in the two states. In order that any substance may be alive it must be an organized body, constructed in such a way that its surface plays the double rôle of preventing the basic substance of the body from diffusion into the water which envelops it; and of allowing to pass into and out of the bodified substance so much and no more of foreign substances as is essential to the body's peculiar composition and action. The surface of the body is as essential to its being alive as are its internal parts or elements. The concept "to be organized" is essential to the concept "to be alive."

Even when cells are constituents of multicellular organisms they have to be conceived organismally, if viewed as physico-chemical bodies and *while the bodies are still living*. Those who conceive organisms in a strictly elementalistic fashion are not conceiving *living* beings at all. Such persons are biologists in name only. The organismal conception must be invoked for interpreting life phenomena at their very threshold and sooner or later at all levels.

The acceptance of the organismal idea as a supplementary working hypothesis at the physico-chemical level of biology would seem to necessitate its acceptance in all departments of the science. Perhaps this would have been so had the acceptance at this basic level taken place early in the history of biology instead of very late. As things have actually gone, each department is having to discover for itself the incompetency of the elementalistic conception to deal lone-handed with all the phenomena of its special province.³

³ Many biologists who are elementalistic on the whole are yet organismic so far as cells are concerned. Most cytologists would probably assent to a statement like the following: "The orderliness of the chemical reactions (in the cell) is due to the cell-structure, and for the phenomena of life to persist in their entirety that structure must be preserved" (A. P. Mathews, quoted with approval by Wilson, 1925, p. 671). The type of biological thinking here illustrated is able to accept the concept "cell" as a category to which the concept "organization" is essential, but is apparently unable to accept the concept "organism" as such a category when it is applied to a whole living being of large size. To them the concept "a man" seems vague and unsatisfactory as compared with the concept "an ovum."

PROTOPLASMIC PHYSIOLOGY

This is the field of research in which the substances of living beings, the protoplasms, are considered as to their fundamental activities in relation to the organism as a whole rather than in relation to any units (cells, for example) into which the organism may be divided.

Professor C. M. Child and his students and associates are the preeminent cultivators of this field. The central conception in the results has been named by Child "physiological gradients." By means of various methods which indicate differences in rate of the metabolic activities in different parts of an organism, Child has shown that orderly development is associated with differences in rate of metabolism. These differences in rate originate as responses to the differential action of factors external to the protoplasms concerned. According to this conception, physiological axes are quantitative gradients in physiological conditions, the most active region exercising a certain degree of dominance or control over others by means of the transmission of effects of its activity. Axial growth and polar differentiation thus result, blocking out the most common animal form, the "head" end being the end at which stimulation and metabolic activity are the greater. The "tail" end is the region in which these processes are least. The gradation or transition of these processes from end-to-end, or from one region to another in any organism, is what Child designates by the term, metabolic or physiological gradients.

That this is only a special form of the organismal (Child prefers organismic) conception is obvious. So far as the basic phenomena under treatment are concerned neither cells nor any other subdivisions of the organisms come into consideration. The only unit of life is the individual organism, its fundamental unifiedness consisting in the correlation of rates of metabolism in its different parts, this correlation being in turn correlated with the response to and conduction of stimuli which are fundamental attributes of the protoplasms of the organism.

In organisms as we everywhere see them the metabolic rate is correlated with the morphological parts and physiological activities. The aspect of the general conception specially developed by Child⁴ is preeminently dynamic and physiological, much emphasis being placed on the "patterns" assumed by the activities involved.

⁴ Of Child's extensive writings in this field, his recent volume (1924) is perhaps the most important general work. He has also given very recently (1927) an excellent brief popular statement of his views.

GENETICAL BIOLOGY

The organismal conception of the basic structure of living matter and activity is forcing its recognition in the quarter where the elementalist conception has stood most stoutly. We refer to modern genetics, which seeks to analyze into its ultimate elements the aspect of heredity manifested in the sexual method of reproduction. The history of this topic presents an enormous accumulation of tedious discussion and acrimonious controversy about various "ultimate units," at one time or another conceived to exist. The famous gemmules of Darwin and the biophores and determinants of Weismann are conspicuous in these controversial writings.

Beginning about 1900, chromosomes were the units preeminently in favor. More recently genes have almost displaced chromosomes, though invisible to the highest powers of the microscope.

Chromosomes have a secure place in objective reality. For a long time they have had objectivity as real as have fingers and toes. They are a part of the demonstration material for classes of beginners in microscopical anatomy. The more they are investigated, the more their objective reality comes to light. That they are in some measure responsible for heredity no one has seriously doubted for two or three decades. The only question that can be raised is as to the all-sufficiency of the chromosome theory of heredity. Are chromosomal elements sufficient to explain the initiation and building up of the individual adult organism? Stated thus it is doubtful that any competent geneticist would now contend for the adequacy of the theory. A few have said expressly that some other elements, as the cytoplasm of the germ and other cells, play an essential part in development.

The chromosome hypothesis must be supplemented by the organismal hypothesis, as other forms of the elementalist hypothesis are being supplemented by the organismal hypothesis in other subdivisions of biology. The interpretation of the rôle of chromosomes in heredity suggested nine years ago (Ritter, 1919, vol. 1, p. 69) was that chromosomes are elements of cells which have arisen by differentiation for the special function of hereditary transmission, just as the characteristic elements of striated muscle cells have arisen by differentiation for the special function of motion production by contraction. Chromosomes may thus be regarded as initiators rather than determiners in individual development. By this interpretation the germ cells would take their place among the other cells of the adult multi-

cellular organism as organs resulting from differentiation for the use of the individual in the course of its wonted life. This suggestion appears to have met with no favor among workers in genetics. Yet it is hard to see how some such idea is to be avoided, with the increasing acceptance of the organismal conception.

There appear to be more signs of recognizing the necessity for supplementing the elementalist conception by the organismal conception in connection with the gene theory of heredity than in connection with the chromosome theory in its earlier form, despite the fact that the genes are supposed to be located in the chromosomes. Thus we are told by one of the foremost experimentalists (Morgan, 1925), that

heredity deals in its theoretical aspects with discrete units, not with wholes; yet, paradoxical as it may appear, the characters that are inherited must be supposed to be due to the interaction of a large number of heredity units (p. 693). The evidence shows that while each gene may have a specific effect on certain parts of the body, it may also have other effects on other parts of the body. Furthermore, each organ or character is the end result of the action of many genes. In fact, each part may be said to be the end product of the activity of all the genes —each one contributing something to it at one or at many stages of its development (p. 717).

These statements and the frequent reference to the rôle of the "whole genetic complex," especially the statement that the whole genetic complex "is present in every part of the body at all times" appear quite compatible with the organismal conception as we have defined it.

To Morgan it seems paradoxical that although "heredity deals in its theoretical aspects" with discrete units, inherited "characters" "must be supposed to be due to the interaction of a large number of hereditary units." The contribution of hereditary units to the production of the characters of an organism is no more incredible than is the contribution of the nutritial units consumed by the developing organism. The nutritial process is, if anything, more paradoxical, more incredible, than the other. In maintaining themselves in the metabolic processes the genes might be supposed to be merely keeping up the habit of the ancestral line. The general body tissues on the contrary would have to be supposed to do the trick more or less anew, as long as differentiation should continue. This is on the supposition that genes are subject to metabolism just as all other living parts of the organism are. Presumably no geneticist conceives genes to be exempt from metabolism and other processes

definitive of living matter. If conceived with such exemption they fall into the same category as the discarded "ultimate" units of which the Weismannian determinants were examples and would be subject to all sorts of mysterious endowments.⁴ The long era of hereditary units assumed to meet the needs of the process of genesis but just "taken" as though from out the blue sky so far as their structure and nourishment are concerned, appears to be coming to an end, thanks largely to the great work of geneticists themselves. As Frank R. Lillie (1927) has recently pointed out, biology has reached a state in its progress where it will have to face the physiology as well as the genetics of individual development.

On the basis of our conception of all organic activity as implying reacting systems, to which chemical transformation as well as surface interchanges are fundamental, we can make reasonable guesses about the nature and origin of genes. We may surmise that they are the smallest masses into which a portion of the substance of a living organism can be resolved, and still preserve (in the latent state) attributes of the organism. Such resolution of substance would facilitate the reproduction of the individual through self-division. Incidentally, the intermingling of the substances of two individuals in connection with reproduction would be made possible.

On this view the system of genes would be acquired by the individual during its ontogeny just as any of the other systems of organs and tissues are. The ontogeny would then be counted as beginning at the time when the primordial germ cell is differentiated in the early embryonal life of the parent organism. The whole ontogeny of an individual would then fall into two periods. In the first period the individual in the form of a germ cell is undergoing the chemical differentiation characteristic of the species to which the individual belongs. This differentiation is conditioned on the germ cell's remaining a part of the parent organism. During this period the parent is impressing its species characters upon its prospective offspring, the system of genes being the chemical form resulting from this impressment. The second ontogenetic period would be that with which embryological science has long been occupied, and concerning which we know vast numbers of factual details, especially on the morphological side.

⁴ As a matter of fact something of the kind is showing itself. Thus "a gene or factor, then, is a ferment activated by an impulse" (Uexküll, 1926, p. 207). It is not probable that biologists carrying on researches in genetics will find anything useful in such speculations.

This suggestion concerning the origin and nature of genes seems to be in harmony with the fact that every explicit, definitely localized, visible differentiation is preceded by an implicit, more or less diffuse, invisible differentiation. A well-known case is that of the lens-producing ability of much of the amphibian ectoderm at a certain stage of embryonic life, this potency being actualized only where the optic vesicle comes in contact with (stimulates) the ectoderm. These "definitely determined loci (primordia)" as F. R. Lillie (1927) calls the differentiations-in-potentia, must play a great rôle in ordinary ontogeny.

Investigation of the total ontogenetic career of the individual would include not only the primordia of that portion of the ontogenetic career which begins with fertilization, but also of that portion of the career represented by the incipient and maturing stages of the germ cells themselves. These stages would be the ones in which the primordia are called genes.

The difficulty of understanding how the developing parent organism may so act upon its incipient and maturing germ cells as to resolve portions of their material into genes, is by no means escaped by the conception of genes now prevalent among geneticists. For if each part of the completed organism is the "end product of the activity of all the genes," even though "each gene may have a specific effect on certain parts of the body" (Morgan, 1924), some power must be operative other than those possessed by the genes each acting all by itself, to produce the results we actually see. If, for instance, all the genes have some toenail-forming power but exercise it only when the "end product" is to be a toenail—never when that product is to be a salivary gland—what is it that makes these genes exert this rather than some other of their powers?

It will be noticed that this suggestion does not require us to give or even attempt to give a complete, a final, answer to this question. All it requires is recognition that no answer is even approximately adequate if it leaves out the organism. If we try to give an answer in the terms of physics and chemistry, we can do so only by adding to these terms others which never occur in physics and chemistry except as they are borrowed from descriptions of the organisms themselves. In making such efforts we go through a mental activity similar to the physical activity involved in trying to climb a hill by walking in a treadmill. Even conceiving genes as psychoids or entelechies does not escape the necessity of the organismal conception if we ourselves as the conceivers are included in living nature.

Our suggestion also seems to be in harmony with the known fact that, so far as the animal world is concerned, a good deal of reproduction (as in some cases of fission, and in all cases of gemmule formation), involves a measure of structural simplification, that is, of reversal of differentiation. This is technically called de-differentiation and may be accompanied by striking diminution in size. Why may we not look upon the origin of genes as a very extreme stage of some such de-differentiating process as those just referred to but pertaining to an indefinitely long phylogeny instead of to a single ontogeny?

RESPIRATORY BIOLOGY

The processes concerned in respiration with their corresponding structures illustrate the ease with which we may misinterpret a function in so complex an organism as man. In common knowledge the lungs and the portion of the blood system connected immediately therewith are thought of as the organs of respiration. This is so in a sense, but only in a very secondary and subordinate sense. The real seat of respiration is the living tissues of the whole organism. Present-day investigators distinguish between respiration proper, i.e., tissue respiration, and structures and activities accessory thereto, by speaking of the latter as ventilating structures and activities. "The function of circulation and respiration is nothing more than the maintenance of adequate diffusion gradients between the tissue cells and their environments, the tissue fluids of the body" (Gesell, 1925, p. 557). These facts were seen in broad but rather distinct outline by Claude Bernard about seventy years ago. In his *Leçons sur les phénomènes de la vie* this physiologist wrote: "All the vital mechanisms, varied as they are, have only one object, that of preserving constant the internal conditions of life." And the "internal conditions of life" are all those factors in the relation between the tissues of the body and the fluids of the body upon which depend the maintenance of the organism in the living condition, the preservation of its identity and the insurance of its continuance in time.

To avoid giving unwary readers the impression that the problem is simple, another quotation may be given which hints at its enormous complexity. The quotation is from Haldane (1922, p. 384). We read:

The facts collected in the present book show that also as regards hydrogen and hydroxyl ions and free oxygen the composition of the blood plasma in contact with any particular part of the tissues is, and must be, very constant,

and is kept so by regulation of breathing, circulation, kidney excretion, and other physiological activities. Thus oxygen and hydrogen and hydroxyl ions take their place in a strict quantitative sense beside the salts, proteins, sugar, etc., which help to make up Bernard's "conditions of life."

Such statements as these are clear in their organismal implications. Certain facts have come to light through researches, which are the exact opposite to what would be expected in accordance with the elementalist hypothesis taken by itself alone. The "paradox of anoxemia hyperpnea" affords an instance. If there is a shortage of oxygen in the air one breathes, as when he is on top of a high mountain or 15,000 feet up in an aeroplane or balloon, instead of breathing less rapidly than the normal (as, in accordance with elementalist hypothesis, the diminished oxygen action would require), he actually breathes more rapidly.

The efforts to resolve this paradox and various other puzzles connected with the speeding-up and slowing-down of the ventilating aspects of respiration have induced some of the most refined researches known to modern physiology. To follow them is quite beyond the purpose and scope of this discussion, even were we competent for the task. The old explanation that increase of lung-and-circulatory movements is due to an increase of carbon dioxide in the blood, with its resulting effect on the respiratory center, has been shown to be erroneous. The bearing of the newer results on the general conception of respiration is made clearer by the following:

What the respiratory center really reacts to, and what in truth regulates lung ventilation is blood hydrogen ion concentration. It is to preserve blood neutrality, not carbondioxide tension, that the mechanism is designed It is found that it is the hydrogen ion concentration which the organism seeks to keep constant, not the carbondioxide tension. (Quoted by Gesell.)

Otherwise stated, the life of the organism depends on the maintenance of constant hydrogen ion concentration, the modification of its ventilating functions being accomplished by the organism as a response to very slight changes in the blood hydrogen ion concentration, rather than as response to changes in the oxygen concentration of the surrounding air.

Recent work in this field dates chiefly from the fundamental researches by Haldane and Priestley, going back to about 1905. Haldane's interpretation of respiratory phenomena is pronouncedly organismal, at least so far as taking the organism as a unified whole is concerned. It is widely known that Haldane is a professed vitalist.

According to our view the organismal conception does not at all necessitate, as Haldane and many others suppose, commitment to any form of metaphysical vitalism. We suspect Haldane's espousal of the vitalistic metaphysics is due more to certain psychical and epistemological difficulties he has found to beset the elementalistic conception, than to the interpretive necessities of the facts brought out by his researches on respiration.

NEURAL BIOLOGY

From the respiratory function, sharply restricted as to the part it plays in the metabolism of organisms, we turn to the nervous function, sharply restricted as to the part it plays in the body movements of animal organisms.

This function is concerned primarily with making the movements of organisms more effective, partly through increased speed and variety, and partly through completer control and coordination. It is therefore seen to be devoted in a very special way to making organisms more thoroughly organized, more definitively organic. This attribute of the nervous function is generally recognized in present-day research and this recognition is becoming part of man's general education about himself. The work of Sherrington (1906), sufficiently and admirably summarized the state of knowledge two decades ago. Though now rather old, this book has done and is doing much toward promoting the organismal conception so far as the nervous system itself is concerned, for man and other mammals.

The researches of G. H. Parker and his students, ranging so extensively over the animal world and dealing with the function in so many of its aspects, are contributing greatly to the organismal interpretation of the nervous system and its activities as in itself unified, and also as a unifier of the whole organism through its relation to the muscles and other organs of the body.

Finally, there is the work of the neurologist Herrick who has positively adopted the organismal conception as a "working hypothesis" in the sense advocated here. The coordinated efforts of Herrick (1924) and Child (1924), culminating in their companion books, have given us the best example we yet have of the elementalist conception, supplemented and controlled by the organismal conception, dealing with the special group of biological phenomena pertaining to the nervous system.

ENDOCRINOLOGY

The characteristic function of the internal secretions is that of contributing to the achievement and preservation of coordination, balance, and unification among the diverse parts and functions of the body. Investigators recognize that the endocrine glands constitute in the aggregate only a minute fraction of the entire body mass, are widely and apparently indiscriminately scattered through the body, and produce substances so minute in quantity as to be insignificant as building materials or energy yielders for the organism.

It is perhaps not superfluous to append a list of endocrine glands. As usually given they are: the thyroid and parathyroids, located in the throat; the pituitary with its two parts located on the floor of the skull, attached to the under side of the brain; and the adrenals, located in front of the kidneys. The gonads (sex glands), the pancreas, and various other organs are now known to produce internal secretions. The possibility that all cells of the body do this is referred to later.

The indubitable results so far attained concern the coordinating office of the secretions. Internal secretions add nothing to the materials of which the organism is composed, as do the substances produced by cartilage and bone-secreting cells. The name hormone, given to the secretions when their real nature was discovered, tells the story of what they do. This Greek word means "I call, or arouse to action." Hormones are "substances having the property of serving as chemical messengers, by which the activity of certain organs is coordinated with that of others" (Bayliss, 1920, p. 712). A typical hormone is the substance, secretin, produced by the mucous membrane of the small intestine and carried by the blood stream to the pancreas, where it incites the characteristic secretive activity of that organ. This hormone is the messenger between the intestine and the pancreas. Its sole function is to bring about cooperation between these two organs of the body, separated in space and diverse in function, to the common end of digesting food. Since every activity of a living being is a response to a stimulant, each of these organs must receive its appropriate stimulation in order that it may play its part in the nutritive function. The food to be digested cannot stimulate the pancreatic cells into action, for they have no contact with the food. This internal secretion, or hormone, is the agent for that

stimulation. The secretion is "internal" in that the intestinal cells which secrete it discharge it not into the intestine itself, there to mix with the food, but into the blood stream as it comes close to the secreting cells. The secretion is a hormone, or activator, in that when it reaches the pancreas it stimulates the cells whose function is to produce the digestive juice in question.

This internal secretion plays a part similar to that of the electric spark in a gasoline motor. The gas explosion, not the spark, does the automotive work. The pancreatic secretion, not the hormone, does the digestive work. The hormone has no more to do with the actual elaboration of the "juice" in the gland than the spark has with the presence of the gas in the cylinder. From the standpoint of the motor's proper function, the electric spark is wholly secondary, but without the spark to activate the gas nothing would happen. The gas is powerless to explode itself. However ready to secrete the pancreas may be, no secreting would occur unless secretin or some other agent stimulated it. Furthermore, just as the spark of the motor must be produced at the right time and place relative to the particular body of gas to be exploded, so the hormone must be produced at the right time and place relative to the particular body of digestive juice to be discharged from the pancreatic gland.

All the best attested discoveries concerning the part played by the internal secretions in the physiological functions of the organism confirm the supposition that their rôle is exclusively that of stimulating different parts of the organism in their characteristic activities.

This account of the nature of hormones implies that their existence and action are dependent on the previous existence of organic parts to produce them, and of other parts for them to act on. They are not original and basic producers at all, but only helpers. Their existence presupposes an organism one of whose attributes is the ability to produce these substances to meet its own needs. The existence of hormones implies the preexistence of a rather highly elaborated organism. There is no certain evidence of their existence in the simplest animals and it is uncertain whether they exist in the plant world. Yet no one questions that these myriads of hormoneless beings are organisms.

There is a growing tendency among investigators to recognize internal secretory structures not as wholly independent of one another but as constituting a coordinated and cooperating system of structures, the function of which is to secure and to maintain on the

chemical level the proportion and balance of the organs and tissues characteristic of the species to which the individual organism belongs.

It is obvious that this system plays a part in the development as well as in the functioning of the organism. Every organism exhibits during at least a portion of its lifetime forms of activity other than its functional activities, resulting in increase in size and differentiation in structure. The growth and differentiation of a digestive tract or an eye, taking place before there is any chance for them to perform their proper function, are very different activities from digesting and seeing. Internal secretions undoubtedly play a part in these developmental activities. The secretory activity of many classes of cells in any developing animal organism is one of the conspicuous phenomena of embryonal life. Secretion of mucus, and of cuticular and shell materials by the outermost (ectodermal) layer of cells; of inter-cellular substances by connective-tissue cells; and of bony substance by bone cells,⁵ are familiar illustrations. Internal secretions, using the term in this wider sense, undoubtedly play a considerable part in the growth and development of animal organisms as well as in their special functioning. But in so far as they contribute actual material for the upbuilding of the organism, they are functioning as something other than hormones. Accordingly such secretions will be excluded from the conception of hormones as understood in this discussion.

Striking evidence of the office of the internal secretory system is furnished by developing individuals in which certain members of the system have failed, thus giving rise to organisms in which coordination and balance of parts is not accomplished. Physicians come most in contact with such organisms and so have the best opportunities to study them. It consequently happens that some of the most convincing evidence of the coordinative nature of the endocrine glands comes from the medical profession.

It is true that up to the present we really know nothing exactly concerning the intimate process in these reciprocal actions, but in a clinical relation such correlations force themselves unmistakably upon the observer. . . . We may conclude that a harmonious development of the body is not possible without orderly functioning of the ductless glandular system (Falta, 1916, pp. 6 and 15).

⁵ Such activities have given rise to the idea that all the cells of every higher animal, no matter what specific function they perform, also secrete substances of some kind. It is certain that such substances as the antibodies produced by the organism for counteracting the effects of harmful foreign substances play an important part in maintaining the health and integrity of the organism. It seems not unreasonable to extend the idea of internal secretions to all such cases, as is favored, for instance, by Biedl (1913).

The work done in the decade since this was written appears to confirm this statement, so far as concerns the interrelations of the endocrine glands.

Much impressive evidence of the rôle of hormones in development is coming from experiments on animals, especially from experiments on the gonads. Castration and transplantation of both male and female gonads, are producing a mass of interesting though bewildering facts. That the ovary and testis produce hormones which influence the development and the functioning of other parts of the body is proved by ample experimental evidence. The familiar effects of castration of the males among domestic animals are confirmed by scientific investigation. It is found that these effects are largely due to hormones, secreted not by the sex cells themselves but by other cells of the gonads. An experimental method employed in addition to castration consists in transplanting the whole or a portion of the gonads, male or female, to different parts of the body of the same individual, to different individuals of the same sex, or to individuals of different sex. The "feminization" of male guinea pigs by grafting ovaries upon them is indubitable. The experiment was first successfully performed by Steinach (1912). Steinach's experiment consists in castrating male guinea pigs two or three weeks old and grafting ovaries upon them on various parts of the body. In the most successful cases the mammary glands and nipples of the individual treated develop as the animals reach full size, in essentially the same way as do the same parts of gravid females. The feminization includes the "mothering" activities when infant pigs are at hand, these activities going to the extent of yielding milk to the nursing young. This case leaves no room for doubt as to the stimulatory influence a hormone secreted by a gonad may have on the development and function of parts of the body remote from, and both structurally and functionally different from, the gonads themselves.

But all the evidence is by no means so clear cut and easy of interpretation as this. While nothing has been discovered which contradicts the conclusions warranted in this case, many facts prove that the situation involving the gonads and all the other endocrine glands is so enormously complex as to defy complete analysis. The following quotations epitomize the present state of knowledge in this domain:

Many authors in recent years have adopted the view that the sex characteristics depend in reality not only upon the sex glands, but upon the whole internal

secretory system. . . . There can surely be no development of sex characters without all the other organs of internal secretion participating; all these glands are influenced by the internal secretion of the sexual glands, and the latter are influenced by the former, and no doubt these inter-relations between the endocrine glands are of the greatest importance in relation to the development of the sex characters (Lipschutz, 1924, p. 103).

And further from the same work:

The greater the knowledge we possess concerning the dependence of morphogenetic processes on the sex glands, the more complicated the whole problem becomes and the greater are the difficulties to be surmounted. Many apparently contradictory facts are probably due to the practice which is perhaps inevitable, of attempting to simplify our problems arbitrarily, in considering the function of an endocrine gland or of a system of such glands as something acting *per se*; in reality, there are mutual relations existing between all the internally secretory organs on the one hand, and between the whole endocrine apparatus and the other parts of the organism on the other. In physiology we are often compelled to think too morphologically, and we attempt to localize functions in a way which does not correspond with reality (p. 477).

These statements, made by an experienced and careful observer, go to the heart of the problem of internal secretions, and of the much larger problem of the organism as a unified whole. The trend of factual evidence and carefully formed opinion in the field of endocrinology is toward the organismal conception, recognizing that the organs of internal secretion constitute a true chemically functional system. It also recognizes that this system is subordinate to, because only a part of, the organism; and that it exists and functions in a manner which maintains and increases the welfare of the organism.

The internal secretory system might be defined as a system of organs and tissues differentiated as intrinsic parts of each individual organism, the system's office being to achieve and maintain through chemical means the type of morphological coordination and physiological cooperation among all the parts of the individual which characterize the species of which the individual is a member.

Thus far, the organismal trend of interpretive thinking in the field of endocrinology is undoubted. No physiologist doubts that every organ and tissue of a complex animal organism is in reciprocal relation with every other organ and tissue. Thus far all physiologists are probably organismal. Very few have moved to the more advanced position which holds that the organism is individualized and unified in such a way as to give it as one whole a measure of determinative power for its own welfare over each of its parts.

The organism as a whole and each of its parts act determinatively on each other, just as the blood system and the nervous system act

determinatively on each other. Genuinely voluntary activity may be taken as the highest form of the organismal phenomenon, the muscular movement involved being its most obvious physical aspect while the metabolic processes of the nervous system involved are its most recondite chemical aspect. That the organism as a whole determines to some extent the chemico-neural actions involved in rationally conscious actions, is the extreme manifestation of the unitary action. The organismal conception, as upheld in this essay, maintains that the evidence available makes it highly probable that the system of internally secreting glands and tissues is the animal organism's chief agency for acting determinatively on its parts at the chemical level of its existence, in behalf of its own welfare so far as this depends on the coordination and cooperation of its parts.

The human species is the most complex of all organic species in its nervous system. There is abundant evidence that coordination in structure and function is a cardinal principle in the evolution of organic beings. We are therefore justified in supposing that the endocrinial system of the human species is complex proportionally to the complexity of its nervous system. Since the endocrinial system is a means by which the organism acts chemically on its parts, the human organism might be expected to surpass all others in power of chemical self-regulation as much as it surpasses all others in its powers of neural self-regulation.

This relation between the nervous and endocrinial systems in the self-regulation of animal organisms may have exceedingly far-reaching bearings on human welfare. For does it not suggest that the emotional part of man's nature holds much the same relation to his whole metabolic activity that the rational part of his nature holds to his voluntary muscular activity? This would seem to follow from the fact now pretty well established that emotional life is largely a matter of endocrinial action. Therefore, in so far as the human organism controls its emotions it really controls its metabolism by way of its endocrinial system.

But since it is certain on the evidence of both common experience and technical research that one's emotional nature is controllable to some extent by his rational nature, it would follow that the human organism does have some power of rational, or voluntary, control over its metabolic process. We should expect to find this power in operation when the individual anticipates emotional states through thought and reason. Something of the meaning of this interpretation

for self-education and self-development, should it become generally recognized and utilized, is not difficult to see. The exaggerated and perverted speculations which have been rife in connection with the human endocrinial system are due to wrong ideas about its functions rather than to wrong appraisement of its powers exerted in their proper rôle.

PSYCHOLOGY

It might be supposed that psychology would stand foremost among those sciences in which the definitive phenomena themselves furnish convincing evidence in support of the organismal conception. As a matter of fact, this has not been the case. Our normal conscious lives flow on from minute-to-minute, day-to-day, and year-to-year as unified wholes, with no immediate awareness of bodily elements. But when we have undertaken to deal with conscious life from the standpoint of the nature of our knowledge itself, this familiar truth has been largely ignored. Effort has been directed to the resolution of knowledge into elements comparable with the elements of which morphological and physiological analysis proves the human organism to be composed. By education and habit the body has been thought of as "built up of," or as an "assemblage of" elements, and the effort has been made to think of mind in the same fashion.

The associationist psychology was elementalistic in the strict sense of the term. Advance in positive knowledge of mental phenomena disclosed the inadequacy of this hypothesis. The theory of psycho-physical parallelism is also essentially elementalistic. The conception thus designated is a technically refined form of the much older doctrine of the essential separateness of Mind and Body. No more disastrous error of common knowledge has ever been carried over into scientific knowledge than that of the supposed separableness of inseparable things in this particular case. The case from the history of science which perhaps comes nearest to this in unfortunate influence on human thought was the astronomical theory which made the earth the center of the universe. Further development of the theory of emergence in evolution, and of the concomitant organismal theory of structure and function will put an end to the domination of all forms of disruptive dualism as between mind and body.

However, so far as our information goes, no professional psychologist has yet fully espoused the organismal conception of living beings, though C. Lloyd Morgan's advocacy of emergent evolution

seems to commit him to the conception. Emergent evolution and the organismal conception applied to living nature are the same thing looked at from different directions. "Emergent evolution" is what that "same thing" is called when the origin and development of living beings are the central interest, while the "organismal conception" is what it is called when their morphology and physiological functioning are considered.

The group of American psychologists constituting the school of behaviorism, are apparently elementalistic, despite the strong emphasis placed by some of them on the organism as a whole. The biological groundwork of the school seems to be that of the biology which conceives the organism as "built up" of cells instead of the biology which recognizes that the organism in its developed state is resolved into cells.

These students have become much impressed with the pervasiveness and potency of the interdependence and coordination of structures and activities within the individual organism. But there is no evidence that they see in the organism as a whole a determinative factor for the structure and activities of its own elements.

The great significance ascribed by behaviorists to the conditioned reflex and to the more general ideas of conditioning and being conditioned, appears to imply the conception of stimuli and responses originally elementary and unorganized in a sense quite unwarranted by the foundational biology of the processes. The "unconditioned responses" recognized by these students as the forerunners of "conditioned responses" appear to be very different things in their view from what biologists of the most penetrating knowledge can allow them to be. Perhaps the crux of the matter here is that behaviorists, regarding themselves as psychologists, are trying to use the idea of the conditioned reflex in ways in which physiologists, with whom the idea originated, would not try to use it.

Indeed it seems to us this surmise is justified by the treatment the idea received at the hands of Pavlov himself, the originator of the idea (Pavlov, 1927). Two considerations especially recognizable from a general inspection of this work of Pavlov appear to support the surmise. One is the fact that Pavlov's expressed idea of unconditioned reflexes accords them, by clear implication, a place in the organic scheme which by somewhat obscure implication behaviorism denies them. Thus in suggesting that "unconditioned reflexes" as he conceives them might be called "species reflexes," while his "con-

ditioned reflexes" might be called "individual reflexes," Pavlov (p. 25) allows due weight to racial and to hereditary factors. These factors are on the contrary almost eliminated from the realm of animal activity in behavioristic psychology at least so far as Watson, the chief protagonist of the school, is concerned.

The other of the two facts above referred to stands out sharply in the title of Pavlov's book. "Conditioned reflexes" are investigated relative to the "physiological activity of the cerebral cortex." That is to say the major interest of the researches appertains to a portion of the animal organism which hardly figures at all in behavioristic psychology. For instance the cerebral cortex is not mentioned, apparently, in one of the latest and fullest of Watson's expositions (Watson, 1924). The brain is mentioned but hardly more than that. Apparently the behaviorist's effort to eliminate "consciousness," "mental" and similar terms from psychology will, if successful, require the cerebral cortex to be eliminated from anatomy and physiology, or at least that some function be discovered for it quite different from that ascribed to it during the whole era of the scientific study of man and the higher animals.

The idea of conditioning as behaviorists use it amounts to rehabilitation of the old associationist psychology by connecting it more definitely with stimulations and responses and the structural groundwork of these than the older form of the conception tried to do. The defect in both forms of the conception is the assumption of innumerable elements that come from somewhere, nobody knows exactly where, and that get into connection somehow, nobody knows exactly how; but once in existence and collected together, they become little by little really associated with one another (older theory) or they come thus to condition one another (newer theory), and thus compose mental life. Sensations and ideas were the independent elements out of which mental life was built up, or aggregated by the associationist theory. Responses to stimulations are the independent elements out of which mental life is built up, or aggregated, by the conditioning theory.

The leaders in the new movement known as *Gestalt-Psychologie* have gone far in the direction of an organismal conception of living beings. The *Gestalt* ("configuration") is the accepted English equivalent) which is the central idea of this theory, is at its very foundation a *composed* something. The theory makes no effort to start off with simple elements, a primal need and task of which is to

get into composition. The basic parts of the basic composition are "qualities" along with their concomitant "background." "Our characterization is, then, this: That the first phenomena are *qualities upon a ground*" (Koffka, 1925, p. 131).

So far the form, and probably the substance, of this theory are clearly organismal. More information as to the nature of the qualities and of their background is lacking. The words just quoted say that qualities and background together are "first phenomena." But a biologist studying animal activity is obliged to ask, "Phenomena of what?" Any satisfactory answer to this query must include recognition of individual organisms as both the "background" and the qualities of the theory. One looks in vain for such recognition. The new conceptions introduced, we are told, "are the simplest mental configurations" (*ibid.*, p. 131). But no definite conception is possible of a mental configuration as distinguished from the activities of an organism.

The *Gestalt-Theorie* may be characterized as an aborted organismal theory, the abortion appearing to be due to lack of an adequate biological foundation, especially in the realm of metabolism, for the thinking of the Gestaltists.

Being more interested in and occupied with the minds of organic beings than with the beings themselves, configurationists have neglected to notice that the term "mind," so far as it has true scientific standing, is a far-gathered abstraction from the activities of living individual beings. All that would be necessary, so far as formulation is concerned, to change the abortive Gestalt theory into a full-rounded organismal theory, would be to substitute for the term "Gestalt" the term "organism," and recognize that the "mental background" of the theory is the organism's general responsive state, while the "qualities" are special responses, or activities, within this general state. We do not see anything in the illustrative and factual material appealed to by configurationists that is not readily amenable to such a change. Take the newborn child in its relation to light, utilized by Koffka: "We ought not to say that the child sees a luminous point; but rather that the child sees a *luminous point upon an indifferent background*" (p. 131). What, one wonders, is the "indifferent background" upon which a child sees a luminous point other than so much of the child organism's total ability to respond to stimuli as is necessary to make the particular response to the particular stimulus (the luminous point) stand forth as such in its conscious life?

A few psychiatrists, notably Boris Sidis, William A. White, Adolf Meyer, and Stewart Paton, have shown marked tendencies toward an organismal conception of human beings. Practitioners in this field are in a specially favorable position to think organismally since they are faced with the reorganization of disrupted activities of individual organisms. Perhaps to no other class of observers is it so obvious that the only access to human minds is through the activities of individual human beings.

While permanent health and progress in all departments of biology are conditioned on the adoption, by students in these fields, of the organismal conception, permanent health and progress in psychology are conditioned thereon in a special sense and measure.

THE CONCEPTION IN THE SCIENCES OF INANIMATE NATURE

The preceding sections of our essay have shown that living nature is revealing evidence of unifiedness among natural forms and forces. Living nature is not alone the source of such evidence. Some attention to similar evidence from not-living nature is incumbent upon us.

Some of the evidence from this source is most striking and pervasive. It must have gripped man in his practical life and enthralled him in his imaginative life at almost the threshold of his career as man. The reference here is to man's early perception of his own personal relation and that of his home country to the sun and other heavenly bodies. We must suppose that man's most primitive consciousness of these phenomena contained some consciousness of a measure of orderliness in the changes that make up the continuity of his life and of the ensemble of things upon which his life depends. That day follows night and night day in apparently endless though varying succession is as obvious as that there is such a period as day and also such a period as night. And similarly as to the years with their seasons.

It is impossible for me⁶ as a human being and professional naturalist to estimate the influence such ideas as that of mountain-making through sedimentary deposits on sea bottoms adjacent to land masses have had on my thinking. Such ideas have had little share in shaping scientifically the organismal conception as it now stands in my mind. Imaginatively, I suspect they have been very potent. At any rate, the little I have learned and am able to understand of the modern theory of isostasy has appealed to me strongly from this standpoint.

A realm of speculation into which I⁶ have ventured rather frequently during the last fifteen years is that created by the doctrine of a primal chaos. Despite the fact that eminent scientists of inanimate nature endorsed it, I have never been able to find any clear meaning in this grand old Hebraic-Miltonian idea. It is an unquestioned fact that our own lives, our knowledge processes by no means excepted, are dependent upon the orderliness of the general scheme of things. How, I wonder, can we even ask intelligently whether there was a time when there was no order whatever? I am unable to put to myself a meaningful question as to the possibility of an absolutely orderless state or even some sort of order absolutely different from that in and through which I live and ask and try to answer questions. There are persons who believe themselves able to do what I am unable to do in this matter. When they undertake to tell me what they can do and how they do it, I always find myself unconvinced of their success.

To put words together to say that the order of nature as we live in it and know it developed from a condition or state absolutely devoid of such order, would be like putting words together so as to make them say that three-angled triangles developed out of two-angled triangles. But speculation of this sort is hardly more than byplay, now that men well equipped to grapple with the question as to the extent of order and system there is in inanimate nature are showing us that these actually do extend as far as trustworthy knowledge-getting has yet been able to go. They have found no sign that beyond the limits reached there exists a realm absolutely devoid of or absolutely different from such order and system. Of several workers in this general realm whose conclusions appear to be of this sort, Whitehead (1925) is the only one to whom we have space to refer.

The theory which Whitehead develops he names the "theory of organic mechanism" (p. 112).

"The doctrine which I am maintaining," writes Whitehead (p. 111),

is that the whole concept of materialism only applies to very abstract entities, the products of logical discernment. The concrete enduring entities are organisms, so that the plan of the whole influences the very characters of the various subordinate organisms which enter into it.

That the concept "matter" is something very abstract and a product of "logical discernment" is a conclusion which a good many scientists have reached by different courses of reasoning. One of

⁶ W. E. R. speaking.

these courses is illustrated by an effort of the senior author made some years ago. I wrote:

We students of nature all find in actual practice that matter is always "matter of" some obvious, easily seen, and handled body. No laboratory or museum so far as I have seen or heard contains a specimen of raw, pure matter. . . . Consequently if we never find any matter elsewhere than in bodies, and if we are never able to resolve a body into pure matter, then, it would seem, pure matter is non-existent so far as observational knowledge is concerned; and, practically, the phrase "dematerialized matter" would be synonymous with "debodyed body." But all bodies are partly sensible, that is, recognizable by our senses, or would be if our senses were sharp enough. So I see no escape, psychological or logical, from the conclusion that the words "dematerialization of matter" are, not sarcastically or ironically, but literally *non-sense* (Ritter, 1918, p. 60).

This statement recognizes that although "matter" is an abstraction, and hence incapable of serving as the basis of an objectively realistic philosophy, yet such recognition does not imply that there is no basis for such a philosophy. The implication is that the necessary abandonment on logical grounds of materialism as the foundation of a world view, does not mean a necessary adoption, on logical grounds, of some form of pure subjectivism, or at least of anti-naturalism (e.g., vitalism) as the basis of such a view. The recognition of "bodies" as the source of "matter" is entirely accordant with a world view having an objective basis. If we define matter as that of which bodies are composed, and define bodies of which we ourselves with our mental processes are examples, as things which we can recognize by more or less constant groups of sensible attributes, and can distinguish from one another so definitely as to enable us to count them, we have a sound basis for the interpretation of an immense range of the experiences of life. Whitehead's great service in this realm is to have brought his ability as a mathematical physicist, his knowledge of the history of thought, and his care and skill in using the instruments of language and logic, to the task of gaining a world view which we recognize to be more coherent the better we understand that view and the longer and more successfully we live in the world thus revealed.

A very important aspect of his achievement is summarized in the paragraph from which the quotation given above is taken:

In the case of an animal, the mental states enter into the plan of the total organism and thus modify the plans of the successive subordinate organisms until the smallest organisms, such as electrons, are reached. Thus an electron within a living body is different from an electron outside it, by reason of the plan of the body. The electron blindly runs either within the body or without the body;

but it runs within the body in accordance with its character within the body; that is to say, in accordance with the general plan of the body, and this plan includes the mental state. But this principle of modification is perfectly general throughout nature, and represents no property peculiar to living bodies (*ibid.*, p. 111).

Some biologists are somewhat piqued by the serious proposal made by Whitehead to call all sorts of existences, atoms and molecules not excepted, *organisms*. Some scientists are quite willing to speak, in a half-metaphysical sense, of atoms and molecules as having life, especially psychic life; but they feel it is going too far to talk about them as organisms. We share this latter feeling somewhat, but not from any sense of sacrosanctity of the term *organism*. It is rather a question of whether the organization of an atom has enough attributes-in-common with the defining attributes of the simplest living being to warrant admitting the atom into a class of natural objects which has been so long and usefully recognized as has that of organic nature, in contrast to inorganic nature. We have reached a stage in our interpretation of nature wherein we ought to rid ourselves of the supposition that any part of nature is really unorganized, or inorganic; but this is very different from saying we are no longer justified in distinguishing between not-living, or inanimate nature and living, or animate nature. We need to recognize that all nature is organic, but that one vast subdivision of it is composed of inanimate organic beings while another vast subdivision is composed of animate organic beings.

The biological naturalist hesitates about accepting as alive systems organized on the plan of the solar system or the atomic system until there has been discovered something more tangible than has yet been reported connecting the central bodies (sun and proton) and encircling bodies (planets and electrons); and until something resembling metabolism, reproduction, and response to stimuli is made out for these systems.

The difference thus recognized between living and not-living organisms leaves gigantic tasks of exploration and interpretation to biologists. Their methods must be largely their own, regardless of how much use they may make of the methods and data of physicists. We biologists are coming to see the justice of such rebukes as that administered to us by Whitehead for "aping physicists." The biologist, becoming consistently philosophical, inevitably meets problems which the physicist, no matter how philosophical he may be, never meets, sense perception, for instance.

PART II

BEARING OF THE ORGANISMAL CONCEPTION ON
PHILOSOPHY

Heretofore our main interest has been in the progress of the organismal conception, and only secondarily in its workings. Now we are chiefly concerned with its workings and only secondarily with its progress. We are to show (if we can) that this conception must be accepted as foundational to the business of philosophy so far as that business is the critical study of the structure of knowledge, of the knowledge-getting processes, and of the trustworthiness and value of knowledge.⁷

The comprehensive study of nature when man is fully included in nature must be pursued with a mental technique adequate to conceive individual objects (of which the conceiving human being itself is one) and all objects to be so related to one another as to constitute the general order of nature, the universe.

That technique as it is concerned with the natural order as composed of countless particulars consists largely of the familiar processes of noticing, describing, classifying, and interpreting the particulars. These processes have been basic to the natural history sciences. But since objects can be counted and measured, as well as described and classified on the basis of their sensible attributes, quantitative as well as qualitative operations must be included in the mental technique called for. That portion of the technique concerned chiefly with the sensible attributes of objects has given rise to what is often called the descriptive sciences; while the portion concerned chiefly with

⁷ The view here expressed concerning the relation of theory of knowledge to philosophy was developed and this whole essay was written in complete ignorance of the following: "The author believed that epistemology has kidnapped modern philosophy, and well nigh ruined it; he hopes for the time when the study of the knowledge-process will be recognized as the business of the science of psychology, and when philosophy will again be understood as the synthetic interpretation of all experience rather than the analytic description of the mode and process of experience itself" (Durant, 1926, p. xiii). If this needs any change to make it agree with the general position of this essay as to the relation between science and philosophy the substitution of "psychobiology" for "psychology" would about fill the need.

the quantitative attributes of objects has given rise to the exact sciences. Much difference of opinion has arisen as to the relative trustworthiness and efficiency of the two aspects of the technique for gaining information and understanding of the world. The chief aim in this part of our enterprise is to show that the validity and power of the descriptive sciences are at least as great as are those of the exact sciences.

Whenever more than ordinary efforts are made to bring together and correlate numberless bits of information about the world, no matter by what mental technique these were secured, so as to obtain the most general understanding possible, the processes involved in these efforts have been called philosophizing, and the results are usually called philosophy. These efforts, whether under the caption of descriptive science, of exact science, or of philosophy, have this in common: all are wholly dependent on our knowledge-getting processes; and all are designed in one way or another to get knowledge and understanding of the world in which we live. So far everybody agrees.

However, as time has gone on and these efforts have become more specialized and complicated, those students who have been particularly attracted to philosophizing have felt the need of attending to certain facts which those who have been more drawn to the sciences have not felt, very keenly at least. The facts alluded to are those concerning the nature of knowledge itself, and of the knowledge-getting processes.

It has thus come to pass that Philosophy as professionally understood in recent times—"formal philosophy"—has considered itself quite sharply set off from Science. And the matter just alluded to, namely, that of the problem of knowledge has become the point at which Philosophy and Science are most trenchantly separated.⁸

Philosophers are wont to state the difference at this point between science and philosophy thus: Science, they say, digs away to gain knowledge of the world without concerning itself with the nature of

⁸ A striking illustration of this difference between science and philosophy, as scientists conceive the matter, is the following: "All the discussion of this essay has been subject to one explicit assumption, namely, that the working of our minds is understood" (Bridgman, 1927, p. 197). This assumption is so far from justified, according to the teaching of formal philosophy, that this department of knowledge makes the "working of our minds" one of its foremost problems. Psychobiological science cannot admit this assumption. Either the writer of the statement quoted is unaware of the position of philosophy relative to the working of minds, or he considers that position as irrelevant so far as concerns the branch of science treated in his book. If true to a well established tradition of the sciences, he probably regards epistemology as belonging to metaphysics and not amenable to truly scientific analysis at all.

knowledge and the workings of minds in getting it, while to philosophy falls the problem of the nature of knowledge. Science gets knowledge of the world while philosophy probes knowledge itself to determine its nature, its trustworthiness, and its value. A technical term used in philosophy for this special problem is epistemology, or theory of knowledge. There can be no doubt that there is here a very real problem or set of problems. There are two component processes in knowledge-getting both of which are exceedingly common and important. These are our sensory processes, in which our sense organs play a great part; and our mental processes of familiar expression. The first mentioned give us sense-data; the second our ideas, or concepts.

Since both these processes are now known to be connected with and dependent on certain of the structural parts of ourselves as organisms—our sense organs, our nerves, and our brains—the getting of knowledge of them surely falls within the scope of the analytical study of human beings. The investigation of knowledge-getting processes belongs at least as much to science, especially the science of man, as to philosophy. If these two sets of processes in knowledge-getting are to be thoroughly investigated, the science of living beings must take an essential part in the business. It cannot be granted that to philosophers alone falls the task of investigating the "problem of reality," to which a close study of sense-data inevitably leads. Similarly the "critical examination of concepts" cannot be adequately done without much help from science for getting information on certain fundamentals of producing and using concepts.

If it be recognized that the problem of reality, with its involvement in the problem of sense-data and also the problem of concepts must be investigated by science as well as by philosophy, which kind of science, descriptive and classificatory science or quantitatively exact, mathematical science, is best equipped for the investigation?

In 1847 Wm. Whewell referred to what he called the natural history mode of philosophizing. He wrote

.... the mathematical and mathematico-physical sciences have, in a great degree, determined men's views of the general nature and form of scientific truth; while natural history has not yet had time or opportunity to exert its due influence upon the current habits of philosophizing.

And later the same author (Whewell, 1858, p. 164) wrote:

Natural History ought to form a part of intellectual education, in order to correct certain prejudices which arise from cultivating the intellect by means of

mathematics alone; and in order to lead the student to see that the division of things into kinds, and the attribution and use of names, are processes susceptible of great precision. (Italics as in the original.)

Whewell suggested that the descriptions, classifications, and generalizations made by petrographers, geographers, botanists, and zoologists may be discovered to have a basis in the mental life of man and in logic that will make it necessary for philosophers to take them seriously. He dwelt particularly on the fact that those parts of the universe which are easily counted and measured, the stellar and planetary systems, and certain aspects of inanimate nature generally, have hitherto dominated the field of philosophy, so far as philosophy concerns itself with the external world. This he suggested has been brought about in the growth of natural knowledge by the circumstance that the quantitative necessities of all reliable knowledge naturally led man to apply his efforts at knowledge-getting to the quantitatively simplest parts of nature first. The one and only sun and moon with their striking regularity of movement; the few planets moving with almost equal regularity; and the relatively few very bright "fixed" stars set man's counting and measuring propensities into vigorous activity early in his cultural career. As contrasted with these parts of nature, how different is living nature! Who can count the blades of grass in the meadow or trace and measure the flight path of eagle or winged insect?

In knowledge of the objects of celestial nature, description and classification play a minor part, numbers and measurements being almost everything. In knowledge of living nature, description and classification play the major part. Classificatory botany and zoology are indispensable prerequisites to all other subdivisions of these sciences. Quantity is, of course, an attribute essential to living as to not-living nature and plays a rôle in the sciences of both; but individualization and variation are so vastly greater in living nature that measurement of quantity can never play so dominant a rôle in the biological sciences as in astronomy and physics.

Whewell suggested that sheer lack of time and opportunity may thus far have prevented the natural history sciences from gaining as much prestige for the methods on which they chiefly rely as the "exact" sciences have gained for their methods. He questioned whether a "natural history mode of philosophizing" might not win favor sometime that would give it the rank which the mathematical mode of philosophizing has long enjoyed. Whewell's suggestion

appears to have attracted little attention. The senior author of this essay has alluded to it in several of his publications during the last decade; but the allusions have been no more successful than the original in gaining attention.⁹

A major purpose of this essay is to show that although the idea of a "natural history mode of philosophizing" is thoroughly sound, it cannot win full validity and force until the considerations favoring it presented by Whewell have been supplemented by the results of a rigid examination *by science* of the nature of reality and of concepts.

Our seeming invasion of the domain of philosophy is not such in actuality. What we do that has this appearance is wholly incidental to our main purpose, such appearance being due to the fact that hitherto philosophy has claimed and tried to solve foundational aspects of the problem of knowledge which do not fall within the proper domain of philosophy at all and can be solved only by psychological analysis.

The naturalist in the sense of the person who devotes himself to the study of nature in one or more of its departments, as the observing astronomer and the field geologist, botanist, and zoologist, is pre-eminently the one who "accepts the universe." He regards nature as something everywhere and always present and, just as a naturalist, he never questions its reality, as at the same time something other than his knowledge and something in his knowledge. The central motive of his researches is to get information about the universe and understanding of it, as it now is, in the past has been, and in the future probably will be. The naturalist as thus conceived is one to whom "nature is everything that is," in the terse language of Brooks (1909). Had Brooks put into words what is implicit in this statement, he would have said that nature is not only everything that is,

⁹ It is, however, gratifying to learn that a few exact scientists are showing signs of distrust of the supremacy accorded them by tradition, over the descriptive sciences. Thus we read: "It must be admitted that science has its castes. The man whose chief apparatus is the differential equation looks down upon one who uses a galvanometer, and he in turn upon those who putter about with sticky and smelly things in test tubes. But all of these, and most biologists too, join together in their contempt for the pariah who, not through a glass darkly, but with keen unaided vision, observes the massing of a thundercloud on the horizon, the petal as it unfolds, or the swarming of a hive of bees. And yet sometimes I think that our laboratories are but little earthworks which men build about themselves, and whose puny tops too often conceal from view the Olympian heights; that we who work in these laboratories are but skilled artisans compared with the man who is able to observe, and to draw accurate deductions from the world about him" (Lewis, 1926, p. 170).

We wonder if it is too much to claim that the caste system might be eliminated from science by making an essential part of the training of neophytes in all the sciences, adequate training in the mental technique of all knowledge whatever?

but is everything that has been and everything that will be, thus giving to naturalists a satisfactory statement of their conception of nature and, by inference, of naturalism.¹⁰ Although this would be at sharp odds with nearly all the conceptions held by traditional philosophy, it would have a claim to respectful attention, as coming from an actual student of nature rather than from persons who are not such students at all in the proper sense. It is one of the paradoxes of the human intellect that the definitions of nature and of naturalism should have been left so largely to a class of students who have not pretended to devote themselves seriously to the study of nature.

Sense-data, ideas and concepts, the language in which they are expressed, and man himself, the producer and utilizer of these, are assumed to belong to the natural order. The naturalist who takes as his lifework the problem of understanding living nature generally, finds himself committed to trying to understand himself and all his works.¹¹

In the physico-chemical discussion of organisms in Part I we saw that basic to all phenomena of life are systems called protoplasmic if viewed from the structural standpoint, and reactive or responsive if viewed from the functional standpoint. We saw that these systems are so constituted that their continued existence is dependent on their

¹⁰ An obvious implication of Brooks's definition of nature is that there is no such bulkhead separating the works of man from the works of nature as custom encourages one to believe. Everything produced by man is just as much a part of the natural order as is man himself. Houses, mines, churches, and hospitals produced by men are parts of the natural order as much as are nests produced by birds, holes in the ground produced by gophers, and plant galls produced on trees by the sting or bite of insects. Even a cubist's painting of a full moon, which ordinary mortals might take to represent a pink-and-green barn half wrecked by a tornado, is nevertheless as much a natural object as is the moon itself or the painter of it. Does it not occupy space? Will it not fall to the floor if its support gives way? Does it not reflect the light? And has it not a complex physico-chemical composition? Not even the skill of a cubist painter is equal to the task of creating an object which transcends the natural order.

¹¹ In a book (Ritter, 1927) recently published it is pointed out that naturalists may facilitate their attack on the enlarged task which confronts them when man is included therein, by dividing the subject matter into subdivisions, one including the whole of nature outside the individual himself, and the other including everything within himself that is in any way involved in his knowledge and knowledge-getting. The two divisions overlap but in that respect they are subject to the conditions of natural classification generally. *Naïve naturalism* is the name proposed for everything done and known in the first division. For everything pertaining to the other division the phrase *critical naturalism* is favored, though *philosophical naturalism* may be more advantageous in some connections. The first part of this essay falls under the caption *naïve naturalism*; the other part, on which we are now engaged, comes under the caption, *critical or philosophical naturalism*.

The systematic treatment of philosophical naturalism is now well toward readiness for publication as a companion volume to the one just mentioned. The title of the companion will probably be "The Natural Philosophy of Conscious Life."

twofold relation to the surrounding world. One of these relations brings about the basic attribute of living beings known as response to stimulus. "Reactivity" is the term favored by R. S. Lillie. Almost the whole of the vast round of activities of living nature is demonstrably dependent on these responses of living bodies to the outside world. The evidence is such as to make it highly probable that all such activities are thus dependent. The other relation is that through which the living body is maintained in its structure, and is enabled to act, by taking into itself energy-yielding materials from the outside world. By one relation to the external world the living being is incited to action; by the other it is furnished with the materials for its body structure and body energy essential to its action.

Foundational to living systems are surface membranes, or layers which play the triple rôle of partitioning the systems off from neighboring systems and the rest of environment; of being the initial place of the system's responsiveness; and of allowing foreign materials to pass through, inward or outward, according to the system's metabolic needs.

Any theory of knowledge which assumes sense-data to be indispensable to all knowledge, must trace knowledge to specific and unmistakable connection with the metabolic processes of the knowing organism. An examination of the connection between the basic responses and the basic metabolism of the organism and the connection of these with the knowing process itself will discover that all our knowledge is basically such that none of it can be certain in the sense in which we are certain of our own existence, except those portions which are immediately connected with our fundamental metabolic processes. No inferential, i.e., deductive, knowledge can be certain in the sense indicated, nor can the sensorially experiential knowledge of any portion of the external world have greater certainty than can such knowledge of any other portion. All objects of the external world, electrons, protons, atoms, molecules, crystals, hills, lakes, stars, nebulae, trees, fishes, men have exactly the same status in our knowledge so far as reality and certainty are concerned. The reality and certainty of such objects can be neither increased nor diminished, nor can one kind be made more or less certain than any other kind by reasoning about them, whether by formal logic, by mathematics, or in any other way.

Hence it results that the sense-perceptive, descriptive, and classificatory method of getting knowledge especially characteristic of

natural history, and traditionally known as the inductive method, is more basic as to reality than is the inferential, or deductive, method.

Apparently it is the great penetrating and broadening power, rather than the power of giving certainty, which has made the deductive method the dominant power it has been thus far in the history of natural knowledge. Mathematics applied to natural phenomena enables us to see with our mind's eyes objects at much greater distances and of much minuter sizes than we can see with our body's eyes. The only drawback is that the mathematically seen bodies are not objectively certain until they have become sense-data to some extent and by some means. Hypotheses concerning nature used deductively are never proved until the results reached are given objective reality by observation.

THE NATURE OF REALITY

The problem of the reality of external nature is frequently stated as the problem of how objects can be external to the mind and internal to it at one and the same time. Thus conceived the problem involves the old doctrine of our minds as having an existence and reality quite distinct from our bodies, our bodies being regarded as matter while our minds are not matter. The problem as thus stated is an inseparable part of the more general problem of how natural bodies of a particular kind can be so related to all other natural bodies as to make us characterize a particular kind of bodies as alive while we characterize all other kinds as not-alive. There is no doubt that the problem we are here approaching is one of the most puzzling that mankind has ever tried to solve. The philosophers, who have hitherto struggled with it, have depended far more on thinking about it than on searching for and using factual information concerning it. They have been more intent on their thought in connection with the problem, than on their lives in connection with it.

Our relation to external objects in virtue of which we perceive them, and thus get knowledge of them, is through their sensible attributes, or qualities. Such of these attributes as their hardness, their heaviness, their color, their shape, affect our sense-organs, our afferent nerves, our brains, and finally by some especially subtle process make us aware, or conscious of them. By these familiar means we come into possession of vast numbers of sense-data.

Sensible attributes belong to all external objects whatever. They enable us to know all sorts of objects, one kind as well as another. But we are not only sensing and knowing beings; we are also living beings, and our living is as dependent on external objects as is our knowing. From much practice in both knowing and living we have found that not all external objects can contribute to our living. Air, water, and certain parts of some plants and some animals do for us when we take them into ourselves what the vast majority of external objects cannot do. Certain natural objects have attributes which become known to us, not by sense perception, but by our taking them as nutriment. Our total information about them is that we can perceive them sensibly, and also use them in constructing and reconstructing ourselves, and in carrying on our activities. By one of our relations to nature we know nature through our sense organs, nerves, and brain. By the other we know it by way of our digestive organs and assimilative tissues. In other words, all natural bodies external to us have sensible attributes, while only a few have life-giving attributes. So far as concerns the life-giving attributes of bodies which possess them, these bodies are real in whatever sense we ourselves are real. We are what we are in virtue of our ability to convert certain latent realities of some bodies into the actual realities which we ourselves are. One's ability to construct his own nature from portions of nature in general is a basic fact of his reality.¹²

But all our experience of external nature is to the effect that the life-giving attributes of bodies which are nutritial are linked with sensible attributes of the same bodies. We therefore have no ground for doubting the reality of the sensible attributes and so of the bodies themselves even though we do not have any such hold on these attributes as we have on the life-giving attributes. It is probable, however, that the basic metabolic activities involve the stimulus-response duality and hence that the life-giving attributes of nutritial materials do furnish sense-data in ways not yet fully understood by us.

¹² "I am convinced that induction must have validity of some kind in some degree, but the problem of showing how or why it can be valid remains unsolved. Until it is solved, the rational man will doubt whether his food will nourish him." (Russell, 1927, p. 14.) Read in the light of the above discussion, the gist of the comment relevant to this quotation is tolerably obvious: The "rational man" should be far less concerned about the uncertainty whether the food he is about to eat will nourish him than with the high probability (faith) that it will because of the certainty that the food he has eaten in the past is now nourishing him.

We here come upon the old puzzle of how the attributes (more frequently called qualities or properties in later discussions) of bodies are related to their particular bodies on the one hand and to perceiving minds on the other. What, if anything, has the roundness of an orange to do with its yellowness? Still greater is the puzzle of how the roundness and the yellowness of the orange can affect us as they do through our senses and minds. The fact that the roundness and yellowness are certainly in our minds regardless of how they get there; and the fact that they *appear* to be also in the orange whether they really are or not gives rise to the problem of "appearance and reality." Which is the *real* orange, the one in my mind or the one out there on the table?

Instead of the usual frontal attack on this problem via the sensible attributes of objects, it is proposed to ignore these, for the time being. The proposal is to eat the orange and thereby prove it to have attributes quite other than its sensible ones, as "sensible" is ordinarily understood, namely, life-giving attributes—*for me*. In other words by this method I prove the orange to have attributes by which parts of it are convertible into myself. These attributes make the nutritial parts of the orange real if I myself am real, for otherwise by eating and assimilating the object, I should be making something real out of what was previously not real. Since chemical transformation is undoubtedly involved in metabolism, this would be equivalent to ascribing reality to the products of chemical reaction while denying it to the substances which enter into the reaction.

This flanking attack on the problem of reality leaves the special problem of sense perception unsolved but serves to free the general problem of our connection with external nature from the bewildering and disquieting doubt which has so long encumbered it. This freeing is accomplished by the absorption of the special problem into the larger problem arising from our being living organisms and our unquestionable connection with external nature. The mystery of sense-data is only one phase of the mystery which underlies the whole of life and of all existence—if mystery is what one decides to call his feeling about what extends beyond his positive knowledge. The mystery we feel concerning existence is our consciousness of the fact that we respond sensuously to vastly more of nature than we can know through our rational and analytic processes. Limitations on our positive knowledge of the natural order appear to be a necessary consequence of our being only parts and not the whole of that order.

In a very literal sense we *know* things immediately only as we *live* them.¹³

Although we are only exceedingly small parts of the natural order, our connection with that order is so close and peculiar that our existence depends on our ability continually to transform parts of nature external to us into our very selves. Since we can become immediate participants in only a small part of the whole of reality, while we can respond sensuously to much larger parts, a good deal of this sensuous response puzzles us (expressing it mildly) as rational beings; and produces in us feelings which we name wonder, mystery, adoration, terror, dread, loathing, depending on the particular conditions, external and internal, existing at particular times.

Nobody so far as known to us has attacked the problem of reality in exactly this fashion.¹⁴ C. Lloyd Morgan (1925, pp. 19, 185, and 186), has perhaps come nearer doing so than anyone else. He divides the attributes of bodies into those of "intrinsic relatedness" and "extrinsic relatedness." The attributes of carbon in virtue of which it sometimes takes the form of the diamond would, it seems, be attributes of intrinsic relatedness; those of its attributes in virtue of which it combines with oxygen to form carbon dioxide, would be attributes of extrinsic relatedness. Those characters of a body which are due to intrinsic relatedness Morgan calls qualities while those due to extrinsic relatedness he calls properties.

It seems that the life-giving attributes of nutritial bodies would be properties of these bodies in Morgan's nomenclature. Possibly, then, his idea contains by inference the same idea that we are exploiting specifically. Answering his own question as to what the

¹³ See note at end of Bibliography (p. 358).

¹⁴ Speaking of "immediacy" in knowledge, Santayana (1925, p. 685) writes: "But what I meant was that everything immediate—sensation, for instance, or love—emanates from something biological. Not, however (and this is another verbal snare), from the concepts of biological science, essences immediately present to the thoughts of biologists, but from the largely unknown or humanly unknowable process of animal life." Is not the "something biological" toward which Santayana is here reaching this very linkage between the living and the not-living via nutritial substances to which we are calling attention? Perhaps insufficient emphasis has been placed on the point, so brief is the presentation, that the linkage referred to gets into our consciousness *immediately* only by way of our metabolic activities. Conceptually we know the linkage only *meditatively*, exactly as we know any of our physiological processes, muscular contraction, for instance. As to immediacy all these processes are unknowable.

The views outlined in this essay appear to provide a psychobiological groundwork for that "falling in love with the greatness of nature" to which Santayana refers as characteristic of some forms of mysticism, that of Spinoza for example. The responsiveness of man to vastly more of the stimuli of the natural order than his positive knowledge-getting can cope with, or than have immediate life-or-death significance for him, seems to be foundational to his feelings of the mystery of existence, and to constitute the mystical element so deep-rooted in him.

reality of a living system would include by virtue of its qualities of intrinsic relatedness, he says: "It would include space-time-event relatedness as primary qualities; it may include physico-chemical relatedness so far as intrinsic only; it may also include life." Except for the phrase "so far as intrinsic only" this statement might be taken to imply the cardinal idea of this essay. But that exception puts the implication in doubt. The idea that the life-giving attributes of nutritial bodies are immediate and indiscerpible linkages between the living organism and the external world is of central significance in this general conception of the nature of reality. These linkages constitute a complete material nexus between living and not-living nature: between subjective and objective, when the reference is to living nature as differentiated into fully conscious organisms.

While therefore we agree with Morgan's inclusion of physico-chemical relatedness in the organic system, our view does not permit this to be restricted to intrinsic relatedness. The restriction thus placed by Morgan on physico-chemical relatedness may be interpreted as restriction to the metabolic processes in play when organisms feed on their own substances, in the absence of nutritial substances from the outside. But it is a biological truism that this sort of thing is wholly secondary. An animal's ability to live for a while by consuming its own tissues is incidental to its ability to build up those tissues from outside materials.

We have seen that the problem of reality cannot be solved without tracing mental activities into definite connection with metabolic activities. Hence the proposal now made for dealing with the problem makes a positive linkage between one of the foremost problems of formal philosophy and some of the positively known phenomena of biology, those of metabolism. The linkage (sense-perception) constitutes one phase of psychology. Since metabolism is a recognized fundamental biological phenomenon, it follows that the linkage is very definitely *psycho-biological*. But much of our presentation in both Part I and thus far in Part II has consisted of evidence that metabolism, and likewise sense perception rooted in the stimulus-response duality, are phenomena occurring only in organisms. Hence by way of the problem of reality the organismal conception wins a secure place in traditional philosophy.

THE NATURE AND FUNCTION OF CONCEPTS

What are concepts? What is their make-up and locus? What is their function? What is their rôle in the natural order?

They occur in connection with living beings, or organisms, and nowhere else. The most common, most characteristic, connection in which concepts are found is in normal adult human beings, although they almost certainly occur at times and in some measure of definiteness in a goodly number of animal species other than man. But it is in the human species that they flourish in overwhelming variety and completeness. This richness of concepts is dependent on human brains and other parts of the nervous system, including sense organs.

So much concerning the make-up and locus of concepts. What are they for? What do they do?

Our answer to these questions is linked with discussions in Part I of this essay.

Each individual man, woman, and child, is a system (protoplasmic and responsive) organized on the fundamental plan sketched in the first section of Part I, especially. The skin of man and of other animals is the elaborated limiting membrane; the whole body structure within the skin is the enormously elaborated portion of the system inclosed by this limiting membrane. All the actions and impulses to action of the human system, no matter how exalted or trivial, good or bad, are elaborations of the phenomena of living systems.

In this first section we saw that every living organism stands in two fundamental relations to the external world, the stimulus-response relation in virtue of which it acts at all as a living body; and the nutritial relation in virtue of which it makes draughts on the environing world for constructing itself and for a supply of energy for its activities. Incalculably great numbers of things in the external world act stimulatively on the organism, most of which are not available to it either as building materials or as sources of energy for acting. From the vast number of these incitements to action the comparatively few which are nutritially useful must be selected.

So far as the responses to stimulatory agencies in themselves are concerned, the organism gets no clue on which to base decisions whether a particular object will be nutritial or not. Neither touch, muscular strain, smell, sight, nor any other kind of sense-data gives one the slightest information as to whether the orange will nourish or poison him, or be merely neutral. Although the whole of external

nature to which we respond sensorially is equally real to us, and our responses thereto are equally basic as life phenomena in us, yet the distinguishing between those parts of nature which have and those which have not life-giving attributes is fundamental.

The ability to distinguish between different kinds of bodies in external nature on the basis of sense-data is consequently indispensable to any measure of organic existence beyond the very simplest. Such existence is made possible by the fact that different sensible attributes of external bodies occur with great regularity not only as among themselves but in connection with life-giving attributes in such bodies as possess them. It is the regular combination of a certain size, shape, color, smell, which enables one to pronounce a certain organic body to be an orange. Further, the regular combination of these attributes with certain life-giving attributes gives one great confidence that a particular orange now before him in its sensory attributes but not yet tested as to its life-giving attributes, will be found to have such attributes if he eat it.

Now this whole business of dealing with external bodies as we humans are able to deal with them is the sum-and-substance of perceptual recognition, description, and classification, and involves the formation and use of concepts almost as basically as it does the occurrence of sense-data. This involvement, stated in the fewest words possible, is as follows: The incitement of an organism to action (stimulation of it) may produce such an effect on the organism (if it has nerves) as to enable the organism to act somewhat differently and more favorably for itself on a future incitement than it would otherwise have been able to act. This peculiar kind of effect of stimulation is basic to what we call memory. Memory is the first step beyond sensation in the direction of ability to form concepts and to reason; and, in general, to lead rationally conscious lives.

The exact structural counterpart of the enormously varied activities presented by the whole animal world, is presented in the nervous and muscular systems found in that world. Concepts, then, are constructions in and by the organism (specifically through its cerebral cortex) as means for enabling the organism to use most advantageously the motion-producing part of itself (chiefly its system of voluntary muscles) for its own welfare. This being the purpose of concepts the critical examination of them is indeed of the utmost importance, not, however, primarily on behalf of knowledge and understanding, but of right action as judged by the welfare of the organism and its kind.

We have now sketched the essentials of the mental technique by which natural knowledge is acquired. That technique rests on the unified structure and action of the human organism—in other words, on the organismal conception applied to mankind. Furthermore, that technique involves the great complex of operations familiar in the history of man's accumulating knowledge as description, definition, and classification, and particularly characteristic of the natural history sciences.

If we have made good the purpose indicated at the beginning of this division of our enterprise, namely, to show the essentiality of the organismal conception and of the natural history mode of philosophizing, a comprehensive statement of what our results imply as to man's attitude toward nature and effort to understand her, can be made in very brief space.

The results imply that all men should be naturalists, in the sense that they should be sympathetic in their feeling for nature, pains-taking in acquiring knowledge of nature, eager in identifying their whole selves with nature, and critical in examining their own mental and physical processes in order to validate both their feelings and knowledge.

Two foremost American philosophers, John Dewey (1927) and George Santayana (1925), who announce themselves naturalists, have recently tilted with each other as to what kind of naturalist each is. We venture to suggest that there is a kind of naturalist which neither of these claims to be. That is the kind who devotes much of his time and energy to the study of nature, doing this because of his love for it and faith in it as that through which his own life and all other lives exist and have meaning and worth.

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NOTE (see p. 351).—The ideas and work of Smuts (1926) must be noticed in this connection. The book which this author has given us is a remarkable one—remarkable we think more as a prophecy than as a fulfillment. Perhaps at no point does its prophetic character appear more distinctly than in the chapter "Mind as an Organ of Wholes." A sample of the author's penetrating insight is indicated by this: "Psychologically the duality of Mind is best expressed in the Subject-Object relation which is fundamental for Mind." (p. 238). This, taken with the perception, expressed in various places, that the double aspect of mind thus indicated involves a "metabolism of a higher order" the understanding of which is impossible apart from "Biology and animal and human psychology," leaves no room for doubt that Smuts's general idea is, taken broadly, accordant with that which we are trying to work out in some detail.

AGE AT PARENTHOOD, ORDER OF BIRTH,
AND PARENTAL LONGEVITY IN RELATION
TO THE LONGEVITY OF OFFSPRING

BY
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UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 15, pp. 359-375

Issued September 12, 1928

UNIVERSITY OF CALIFORNIA PRESS
BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS
LONDON, ENGLAND

AGE AT PARENTHOOD, ORDER OF BIRTH, AND PARENTAL LONGEVITY IN RELATION TO THE LONGEVITY OF OFFSPRING

BY

S. J. HOLMES

The present paper deals with a number of topics concerning which our knowledge is very inadequate. For information on longevity one has to depend upon records which have usually been compiled without any reference to this subject, and for answering many questions such data are apt to prove unsatisfactory. For the student of the biological trend of civilized mankind no information could be of greater value than full and accurate vital statistics of individual families. Did we possess an abundance of such family histories several important problems would be comparatively easy to solve. As it is, we have to depend upon the relatively imperfect material that may be available.

In endeavoring to obtain information on the problems here dealt with I have made use of the statistics on royal families contained in Allström's Dictionary of Royal Lineage. This work aims to give dates of birth, death, and marriage of all members of royal families from the earliest times down to the twentieth century. It is a monument of industrious compilation of dry details, but it has the peculiar merit of containing facts in regard to a class of the population which is economically well situated and in which some of the environmental factors affecting vital statistics are less variable than in the rank and file of ordinary humanity. Unfortunately the data are often incomplete. Not infrequently there is no information upon the age of death of the younger members of a family, and the records of infant mortality are very unreliable. The imperfections of the material became more apparent as work on it progressed, but there are several conclusions which may be drawn from it with some assurance of probability. On a few topics the information was supplemented by data compiled from several volumes on American genealogy.

AGE OF PARENTHOOD AND LONGEVITY OF OFFSPRING

The relation of longevity of offspring to age of parents at birth of offspring was discussed in a previous paper by Miss Wilson and the present writer, but only a part of the material in Allström's work was employed. We limited our study to those families having four sons who lived to adult age, in order to obtain material comparable to that investigated by Mr. Caspar Redfield, who contended that sons coming from older parents lived on the average longer than those coming from younger parents. Our study yielded no evidence of any significant relationship between longevity of offspring and age of parents when offspring were born. In the present paper I have included fuller data and have worked out coefficients of correlation between all offspring whose ages could be ascertained and the ages of both the father and the mother when the children were born. Ages of both parents and children were broken up into five-year intervals and the mid-age taken as representing the average age of the group. In all there were 4177 cases in which the age of offspring could be correlated with the age of the father at the birth of his child.

In dealing with children of ages 0 to 4 inclusive, it would be obviously wrong to take year 2 as representing the average age of the group since many more children die before the second year than in the third to the fifth year. One cannot do better than to make an approximate estimate of the average age of this group. In Glover's life tables of the United States for 1910, it is shown that the number of children dying in the first half of the first year is slightly more than those dying in the remainder of the quinquennium (8592 per 100,000 born as compared with 7521). Accordingly in all of our work with ages of children we have centered the mortality of the first five years at 6 months instead of at 2 years of age. This is probably as good an estimate as could be made on the basis of a simple number. In fact, the differences in the correlations caused by centering early mortality at 2 years and at 6 months are very small. In the case of the correlation between longevity of children and the ages of fathers at the birth of the children, the difference due to centering early mortality at 6 months instead of 2 years was only .00022, which is a quite negligible amount. The correlations between longevity of children and age of mother when the children were born were affected by only .0017, which is also a negligible amount.

The correlation between longevity of children and the age of the father when the children were born (or what we may for convenience designate as the age of fatherhood) was found to be $.02929 \pm .0103$. Taken by itself this might seem to be a significant correlation since it is nearly three times its probable error. On account of the relative incompleteness of the data on ages at death of minors in the material dealt with, the correlation was calculated between age of fatherhood and the longevity of those children who had attained 20 or more years of age. This correlation proved to be less than the previous one, namely $.0161 \pm .01113$, a correlation which, although positive, is not significant in relation to its probable error.

The relation between age of motherhood and the longevity of offspring (including all ages) proved to be actually negative, namely $-.0108 \pm .01203$. The relation is obviously quite non-significant. Taking only the children who lived to be 20 or more years of age, the correlation became positive but infinitesimal, i.e., $.00006 \pm .013$.

On the whole, the fuller data on the relation of age of fatherhood to the ages of all children dying when over 20 years of age confirm the previous conclusion of Miss Wilson and myself that no correlation of a significant kind exists between the longevity of children and the ages of fathers at the time the children are born. And the same conclusion applies to the ages of mothers.

ORDER OF BIRTH AND LONGEVITY OF OFFSPRING

The relation of order of birth to longevity in royalty was also considered briefly in the paper by Miss Wilson and myself but only a limited part of the data in Allström's volume was used because we confined ourselves to families having four adult sons. In the present paper, I have included data on all the individuals concerning whom the ages at death could be obtained. Despite the apparent simplicity of the problem its investigation is beset with several pitfalls, as a perusal of the literature on other studies of the subject will abundantly reveal. Taking the birth of a lot of individuals coming from families of different sizes involves possible errors due to the correlation of small family size with defective vitality of parents. Many families are small because the fathers die early or the mothers are physically unable to bear more children. Again, while large families, as the studies of Bell indicate, are an index of parental vitality, there is a complicating factor due to the frequent association of large family size

TABLE I

AVERAGE AGE OF OFFSPRING ACCORDING TO BIRTH RANK IN FAMILIES OF DIFFERENT SIZES

with poverty and in some cases with mental inferiority, both of which tend to enhance the death rate. In order to avoid as many sources of error as possible, I have calculated the average longevity of offspring in relation to order of birth for families of 1, 2, 3, or more children. I have included only families in which all the children belong to the same father and mother. This excludes the cases of second and third marriages in which a man usually marries a woman younger than his previous wife at time of death, thus complicating the data on the effect of parental age upon offspring. It would also exclude cases in which the birth order of the child was different in relation to the two parents. Offspring dying a violent death were excluded since their longevity would have no biological significance. The results are given in detail in table 1.

An inspection of the results makes it apparent that *within each group of families of a given size* there is little indication that the members of any birth rank live longer than those of any other. This result again bears out the conclusion drawn by Miss Wilson and myself on a smaller sample of the same material consisting of those families where there were four sons to reach adult age. The numbers dealt with, however, were not sufficiently great to reveal anything more than a very decided trend if it existed.

In order to ascertain what relationships were indicated by the data as a whole, order of birth was correlated with longevity of children. The outcome of this rather laborious estimate gave a negative correlation of $-.09047 \pm .01215$. This means that children of higher birth ranks do not live so long on the average as those of lower birth ranks. The correlation is a weak one, to be sure, but it is over seven times its probable error and is therefore not due to mere chance. The result seems not quite in harmony with what might be expected on the basis of the rather low positive correlation between the age of fatherhood and the longevity of offspring. Children belonging to later birth ranks are, on the whole, born to fathers who are older (when the children are born) than the fathers of children of earlier birth ranks. This is of course self-evident when we consider each individual family, for a father must be older when his second child is born than he is at the birth of his first child. But the proposition is not *necessarily* true when we lump a lot of individual families together. It would then be quite possible for fathers of second or third-born children to average younger when the children are born than fathers of first-born children. If fathers of large families marry early, and fathers of small families marry late, and if there are many of the latter class as compared with

the former, the late average age of fatherhood of the parents of small families plus the early ages of the few fathers of large families, might give a higher figure than the average ages of the fathers of the later-born offspring. To a considerable extent these conditions represent actual tendencies. In general fathers of large families marry young. Those marrying late tend to have families which are relatively small and there are more small families than large even in royalty.

In order to test this I selected a random sample of 62 families taken about equally from those which were large and those which were small. The average age of the fathers at the birth of the 62 first-born children was 30.30; at the birth of the 36 second-born children it was 30.06, and at the birth of the 28 third-born children it was 31.18. In this particular group, therefore, the fathers of second-born children actually averaged younger than those of the first-born. We thus have certain factors tending to offset the very natural tendency for age at parenthood and birth rank of children to go together. Whether these factors are responsible for the lack of agreement of the correlation between age at fatherhood and longevity of offspring and the correlation between birth rank and longevity is uncertain. Too much weight cannot be given to the first correlation, however, since it is not quite three times its probable error.

The tendency for the earlier-born children of a family to live longer than the later-born was tested in another way by comparing the age of pairs of brothers, taking the earliest and latest-born male members of each family in order to have individuals separated as far as possible as to period of birth. Beeton and Pearson found in investigating the longevity of pairs of brothers that the earlier-born brothers tend to outlive later-born brothers. The part of their data which was furnished by the pedigree records of the Society of Friends was more complete than the data furnished by the peerages and the usual family histories, but how full and accurate the records really were we do not know. By comparing the ages of members of pairs of brothers or of sisters living to age 20 or over, it was found that "the elder adult sister and adult brother live on the average 4 years longer than the younger adult sister or brother." The interval between the birth of the older and the younger member of a pair varied from one to 29 years. In order to ascertain if difference in longevity was related to length of interval between births, the authors made a correlation between these intervals and the differences in duration of life between the members of a pair, counting the excess of longevity of the older member as positive and that of the younger member as negative.

The correlation between the intervals at birth and differences of longevity was for adult brothers $.1062 \pm .0206$, and for adult sisters $.1201 \pm .0246$. For all brothers the correlation was $.1096 \pm .0141$, and for all sisters $.1352 \pm .0165$.

From a study of these data Beeton and Pearson draw the conclusion that "a brother born 10 years before another brother has probably 7 years greater duration of life; a sister born 10 years before another sister has probably about 6 years greater duration of life." This is a very important conclusion if it concerns a real biological tendency.

The material at my disposal was hardly adequate for testing this conclusion in a thorough way so I limited myself to comparison of those pairs of siblings whose births were separated by the greatest number of years. Correlating intervals between births with differences of ages at death, and counting the excess in favor of the earlier-born as positive and that in favor of the later-born as negative, I obtained for 501 pairs of brothers over 20 a value of $r = .0980 \pm .0268$. This is a little over three times its probable error and might therefore be considered significant. Correlations worked out in the same way for 339 pairs of sisters over 20 gave $r = .0106 \pm .03554$. In the latter case the results cannot be held to indicate any real correlation. In royalty the data on ages of females are less complete than those for the ages of males, and there are several indications that they are considerably less reliable. The average age of the older sisters living over 20 was 52.737 and of the younger sisters 52.958, but the older sister lived the longer in 165 cases and the younger lived the longer in 161 cases, while both members of 9 pairs died in the same year. The data on brothers and sisters may be summarized as follows:

	Brother	Sister
Average age of oldest.	52.105	52.737
Average age of youngest	50.375	52.958
Standard deviation of intervals between births	4.967	4.814
Standard deviation of intervals between deaths	24.040	23.307
Correlation between birth intervals and longevity	$.098 \pm .0268$	$.0106 \pm .0355$

It is of interest that the conclusions of Pearson and his colleagues are supported by the results obtained by A. G. Bell in his valuable study of the genealogy of the Hyde family. Bell found that in 2756 cases where the longevity of the offspring and the age of the father when the offspring was born were both known, the longevity of the

children decreased as the age of the father increased up to the period of 50 years and over, when there was a slight increase in the average age of offspring. The number in the latter age group was not large and the increased age of the offspring was well within the range of chance fluctuation. Bell found that a similar reduction of longevity went along with an increase in the age of mothers at the time of the birth of their children, there being over 10 years' difference between the ages of the children of the oldest and of the youngest mothers. According to Bell, "youth is a condition favorable to the production of long-lived offspring."

The interpretation of these results has long puzzled me. It is uncertain whether the explanation of the findings is to be sought in genetics, physiology, social customs, epidemiology, or some kind of bias affecting the compilation of statistics. In royalty especially there is not only more carelessness in recording the data on females, but also in giving data on the later-born members of a family. I have endeavored to get some clue to this latter bias by compiling the percentages of cases of uncertain age among the members of different birth ranks. These are given in the following table for the several categories of order of birth:

RELATION OF ORDER OF BIRTH TO INCOMPLETENESS OF DATA ON AGE

Birth rank	1	2	3	4	5	6	7	8	9	10										
Percentage of uncertain age	15	93	31	46	33	98	87	87	39	72	40	07	38	54	44	12	48	31	43	04

These figures are drawn from the data from which table 1 was compiled but they were not included in the table, which is limited to individuals concerning whom the ages at death were given. They show clearly that data on ages of children become less complete as the birth rank of the child increases, and that the records of the first-born are given with very much greater fidelity than those of the second or any subsequent birth rank. In royal families there was naturally more concern over the first-born than the later-born, and this doubtless led to more complete and accurate records in regard to the earlier members of the family. Since the children dying young are the ones whose ages are most apt to be unrecorded, one would expect that their omission would tend to increase the average ages of the later-born children. This statistical bias therefore obviously does *not* explain the greater longevity of the earlier-born members of the family.

There is another kind of statistical bias however which would tend to bring about an increase in the recorded longevity of the earlier birth ranks. This is the influence of secular changes in vital statistics. In dealing with statistical material collected at different periods of time, we have to consider how our results may be affected by the changes which occurred while the data were being supplied. Two factors which enter into the present problem are, (1) changes in the average size of the family, and (2) changes in the average duration of life. If while families are growing smaller the average duration of life is being increased (as is actually the case) data on longevity collected during the whole period in question would tend to show a decreasing longevity as we pass from earlier to later birth ranks. No collection of data on this topic is free from the influence of this secular bias, and, so far as I know, no one has called attention to this factor in discussing the relation of longevity to order of birth. The same factor is operative in tending to produce a negative correlation between age of fatherhood and longevity of offspring.

Apparently, however, we cannot account in this way for the tendency of the earlier-born member of a pair of brothers or sisters to live longer than the later-born member. Here we are dealing with members of the same family living in the same epoch. Picking out all possible pairs of brothers in families of various sizes and living at different periods of time would not affect differently the relation of the earlier-born to the later-born members of the several pairs. We have in these intra-family relationships the best indication of a real biological superiority of the earlier-born individuals. But it would be a little dangerous to assume that the superiority of the earlier-born is caused by any inherent biological factors. So far as heredity is concerned the various birth ranks stand at the same level. It might be assumed that mutations injuriously affecting vitality may occur more frequently in the germ plasm of older parents. Age affects the frequency of crossing over in *Drosophila* (Bridges) and the frequency of anomalies in *Habrobracon* (Whiting), but we have no knowledge as to what effect age may have upon the mutation of genes. Wright observed that in guinea pigs the proportion of white in piebald offspring increased with the age of the mother, and that in certain stocks the percentage of offspring with an extra toe on the hind foot markedly decreased with the age of the mother. But these effects are probably merely somatic. We might assume that children of older mothers are handicapped on account of their developing in bodies lacking the vitality of youth. Certainly maternal age has an effect

upon the proportion of stillbirths and the rate of infant mortality, but if later age periods are affected it has never been demonstrated.

It is unfortunate that data for a definite answer to the problems we have been dealing with are so meager. The importance of the problems is obvious. If the expectation of life of human beings is so profoundly influenced by their birth rank as the studies of Bell and of Pearson and his colleagues indicate, the matter is not only of the greatest interest in relation to life insurance but it should affect the reproductive habits of the race. Postponement of marriage and especially the postponement of having children after marriage which is now so widely advocated on economic grounds could not fail to have a bad effect upon the life expectancy of the offspring. Late parenthood with its inevitable reduction of family size would then mean a serious drag upon the vitality of the race whatever it might mean for the trend of our racial heredity.

INHERITANCE OF LONGEVITY

Since the data on royalty furnished considerable material on the duration of life under conditions which were fairly uniform, it was thought worth while to work out correlations for length of life between fathers and children and between mothers and children. The ages of parents and offspring were broken up into five-year groups and the mortality of the first quinquennium was centered at 6 months. Despite the incompleteness of the data on the ages of offspring, the parent-offspring correlations were found to be of much the same order as those obtained by Beeton and Pearson for members of the Society of Friends. In my data the offspring of double marriages were excluded, since with children of the same father there will be some from a mother with short duration of life, and others from a mother with longer duration of life. Such cases would tend to introduce complicating factors in so far as heredity might be a factor in the results. Also deaths from violence were not included as these yield no indication of the natural span of life under usual conditions.

In all data on longevity based upon genealogical records, we have to deal with incomplete data. Some records are better than others, but there is always a considerable percentage of persons about whose age at death no information can be obtained. These are specially apt to be persons who die young as is clearly evident in the data on royalty. In different genealogies dealing with persons living under differing

circumstances the age distribution of the omissions probably varies, since in many cases where the people scatter, data on the deaths of older individuals cannot be obtained. This is apt to occur with American genealogies as contrasted with many of those dealing with more settled communities of the old world. If the omitted records left us with a fair random sample of the population, their loss would not affect the problem. But one can never assume that this is the case. Our mass data on longevity are all subjected to some kind of a bias, and one which varies under different conditions of life. Parent-offspring or fraternal correlations cannot therefore be considered as affording an accurate measure of the tendency of longevity to run in families.

On account of the imperfection of the data on royalty I have limited myself chiefly to working out correlations between parents and children. The correlation between the ages of father and children based on 3169 pairs was $.1430 \pm .01117$, and between the ages of mother and children (2390 pairs) was $.1335 \pm .01355$. While these correlations are weak they are in one case over ten and in the other nearly ten times the probable error and are certainly significant.

If we take out the 0 to 4 age group of children, the correlations become $.1450 \pm .01225$ for fathers and children and $.09044 \pm .0144$ for mothers and children. One might expect a priori that the removal of the group of very young children, among whom it is commonly believed that there is a larger mortality of a purely fortuitous kind, would tend to increase the correlation between the longevity of parents and that of the remaining offspring. While it does this slightly but not significantly in the case of the father, the reverse occurs in the case of the mother. The correlation was reduced in the latter instance by .0418, which is about three times the probable error of the first correlation.

Correlations were worked out also between the longevity of parents and the longevity of those offspring who lived to be 20 or more years of age. The removal of minors had the effect of reducing the parent-offspring correlations to a sensible degree. The correlation between the longevity of fathers and that of children over 20 was $.10523 \pm .0128$, and between the longevity of mothers and that of children over 20, $.09834 \pm .01497$. Even these weaker correlations are very significant in relation to their probable errors. But it seemed at first rather singular that they should be weaker than the correlations which included the relatively imperfect data on the younger members of the family.

For the purpose of making comparisons with royalty I have had data compiled on longevity from several books on American genealogies. These covered periods from the colonial days down to the present century, but the bulk of their data belong to the eighteenth and nineteenth centuries. They represented therefore a group of people living on the average at a somewhat later period than the members of royal families. The correlations for duration of life were $.0687 \pm .0142$ for fathers and children (2250 pairs) and $.1293 \pm .0149$ for mothers and children (1973 pairs). Removing the children in age group 0 to 4 reduced the father-offspring correlation to $.0225 \pm .0157$, and the mother-offspring correlation to $.0879 \pm .0148$.

As shown by the work of Beeton and Pearson and of Ploetz, the mortality of early life is closely related to parental longevity, and Ploetz has shown that this low early mortality is especially apt to occur among children of parents who live to very advanced ages. All these writers attribute this fact to the inheritance of constitutional vigor which is manifested alike in the low death rates of parents and young children.

In order to indicate the relation of parental age to early mortality, I have represented in table 2 the number of deaths and the per cent of children dying for the various age groups of parents from both royalty and American genealogies. In general the data show that early mortality declines as the age at death advances in both the fathers and mothers in each group. The trend of early mortality as the age of parents increases is very similar to that found by Ploetz both in royal families of Germany and in the middle-class population. In another respect my results agree with those of Ploetz in showing a more marked decline in child mortality as the parents come to live into the advanced age periods. This is a striking feature of all of the series which I have tabulated. Apparently the ordinary variations of longevity do not affect the life expectancy of children nearly so much as having one or more parents who die over 75 years of age. The fact that our data on royalty do not show a single death in early childhood among the children of parents who attained the eighty-fifth year is quite significant.

While it has long been recognized that longevity runs in families, our knowledge of the inheritance of this characteristic is very incomplete. There are long-lived families and there are short-lived families, and it is a common assumption that these differences are due largely to heredity. Many cases have been collected in which several members of a family lived to an extreme old age. But while it is probable that

TABLE 2

NUMBER AND PERCENTAGE OF CHILDREN DYING UNDER 5 YEARS OF AGE IN ROYAL FAMILIES AND IN AMERICAN STOCK ACCORDING TO AGES OF PARENTS

Age of parents . . .	10-25	25-35	35-45	45-55	55-65	65-75	75-85	85-
ROYALTY—Fathers								
Number of children . . .	13	107	331	735	789	821	352	21
Number dying under 5 . . .	0	10	29	69	57	71	25	0
Per cent dying under 5 . . .	0	9.35	8.76	9.38	7.10	8.65	7.10	0
Mothers								
Number of children . . .	75	290	338	360	573	456	243	55
Number dying under 5 . . .	16	42	39	36	41	42	16	0
Per cent dying under 5 . . .	21.33	14.48	11.54	10.0	7.15	9.21	6.58	0
AMERICAN GENEALOGIES—Fathers								
Number of children.	2	70	168	268	388	547	560	253
Number dying under 5 . . .	1	17	38	66	77	96	110	35
Per cent dying under 5. . .	50.0	24.29	23.17	24.63	19.84	17.55	19.64	13.83
Mothers								
Number of children.	13	93	145	185	290	408	501	337
Number dying under 5 . . .	5	32	45	36	46	70	96	53
Per cent dying under 5. . .	38.46	34.40	31.03	19.46	15.86	17.16	19.16	15.72

many of these cases exhibit a true hereditary influence, they suffer from the drawback of being selected evidence gathered in relation to the particular conclusion they support. Even if there were no inheritance of long life it would be possible to collect, out of millions of families, a great many cases in which several related individuals lived to an extreme old age. Unless the grouping of these cases is studied statistically in the light of the theory of probability, they furnish little conclusive evidence of the heredity of long life.

So far as I am aware the first adequate statistical evidence of the inheritance of longevity was furnished by Beeton and Pearson. The employment of coefficients of correlation as an index of resemblance in length of life affords a means of detecting relationship amid unselected material, and is almost indispensable for the proper study of the subject. But a parent-offspring or fraternal correlation does not give a full measure of the real "strength of heredity." Moreover the correlations may be rendered spurious to a certain extent through

the influence of environmental factors which affect related individuals in a similar way. The procedure followed by Pearson and his co-workers does not seem to me to get rid of certain sources of error consequent upon secular changes in the material dealt with. Suppose we have data on longevity collected, as Pearson's apparently was, over a considerable period of time. Let us suppose that the average duration of life had been decreasing in the meantime in both parents and offspring, or in brothers and sisters. We should have some pairs living a century or more ago and consequently with relatively low average duration of life and other pairs belonging to a later period when the duration of life was greater. If now we throw all these pairs into a correlation table we should obtain a certain amount of positive correlation even if the duration of life were not inherited at all. It would be rash to ascribe all the observed correlations to this cause alone, but it seems probable that environment should be credited with at least a small part of it.

In order to obviate in a measure the source of error just mentioned and to get some test for a possible spurious correlation in the results obtained from royalty, I broke up the material into centuries. For this purpose I took the length of life of the family, or the period from the birth of the father to the death of the last child, as a basis for grouping. Families whose total life lay within a given century were grouped together and families whose life-span covered the latter part of one century and the early part of the next were classed as an overlapping group. Of course some parent-offspring pairs were obtained which were separated from others by several decades, but the changes in the average duration of life during most of these intervals were relatively small. The endeavor to obtain pairs of individuals living more nearly at the same time would have split the material up into too many groups having only a small number in each.

TABLE 3

CORRELATIONS BETWEEN THE LONGEVITY OF PARENTS AND THE LONGEVITY OF OFFSPRING IN ROYALTY DURING A SERIES OF CENTURIES

CENTURY	CORRELATIONS	
	Father and offspring	Mother and offspring
17th186 ± .0365	.0602 ± .042
17th and 18th.....	.018 ± .033	.084 ± .035
18th.....	.1650 ± .0395	.0125 ± .044
18th and 19th.....	.0598 ± .0313	.04105 ± .0321
19th.....	.0361 ± .0367	.0553 ± .03701

When correlations were calculated for the smaller groups living in the same general period they were found, with two exceptions, to be markedly smaller than the correlation for the group as a whole. The various correlations are given in table 3. The two cases in which the value of r rises above the general level obviously fail to compensate for the low values of all the other groups.

The fact that the correlations for longevity are lower for American genealogies than in royalty may possibly be due to the inclusion of a smaller amount of secular variation in mortality.

AGE GROUPING

Comparison of the data on the distribution of age groups in royalty and in American stock brings out one striking difference. This is the much greater number of individuals among the Americans living to an advanced age. For some reason old people are not frequently met with in royal families. The percentage attaining an age of eighty years is relatively small, and those living to over eighty-five are very rare. On the other hand, in the old American stock represented in our genealogies persons in the eighties are not uncommon.

Records of early deaths are not sufficiently full and accurate to justify any comparisons of infant or child mortality in the two groups. The longevity of parents being usually given, if we limit ourselves to individuals who have qualified as parents we obtain fairly reliable data on comparable groups. In royalty .66 per cent of the children had fathers, and 2.30 per cent had mothers who lived to be 85 or more years of age, whereas in American genealogies the percentages having fathers or mothers at 85 or over were 11.2 and 15.8 respectively. In royalty 2.90 per cent of the children had fathers, and 3.68 per cent had mothers living from 80 to 85 years, and in American genealogies 11.1 per cent had fathers, and 12.48 per cent had mothers living in this age group.

One may assign several more or less plausible reasons for the striking differences in the longevity of these two groups. The Americans whose names get into genealogies probably came in general from a fairly hardy stock, and a considerable proportion of them lived under the relatively wholesome conditions of rural or semi-rural life. They constituted for the most part the substantial middle-class elements of communities whose economic conditions were often fairly prosperous and rarely sank into squalid poverty. Whatever may be said of the

hazards of infancy and childhood, the adults probably enjoyed as favorable conditions for long life as could be found in any other group living in the same general period.

The members of royal families, while freed from the economic hardships which often increase the death rate among the less fortunate sections of the general population, had the disadvantage of living mainly in an urban environment. Their mode of life may not have been particularly hygienic, and although the best medical skill was at their command, it was unable until recent times to do very much for either the cure or the prevention of disease.

CHANGES IN FAMILY SIZE AND DURATION OF LIFE

The data on family size in royalty indicate that up to about the middle of the nineteenth century the birth rate underwent relatively little change. For several centuries one frequently finds large families of a dozen or more children born to a single mother. Frequently also the head of the family married two or three times and produced several children by each wife. The largest number of children recorded as born by a single mother is 21. This distinction fell to the unhappy lot of Elizabeth of Kärthnen whose reproductive career was cut short in 1308 by the assassination of her husband, Albrecht I of Hapsburg.

But while there were many large families in royalty, the average number of children per father or per mother was not high. Their record was considerably exceeded by the prolific stock of our American colonists, among whom the average family, up to the nineteenth century, contained between six and seven children. The average for mothers in royalty was between three and four children, and for fathers, between four and five children. To how great an extent voluntary limitation of the family was practiced in royalty is uncertain.

Statistics on the average duration of life among members of royal families tend to give too high a figure owing to the omission of so many records of those who died young. The imperfect data we have show, as might be expected, that the duration of life increases as we approach the present time. I have given (table 4) the average of longevity for the several centuries from the twelfth down to the period of the last families whose members have all died. This does not cover therefore the latest decades during which there has occurred the greatest increase in the expectation of life.

TABLE 4
FAMILY SIZE AND AVERAGE LONGEVITY OF ROYALTY IN DIFFERENT CENTURIES

Centuries	Average longevity	Number of deaths	Number of children per	
			Father	Mother
19-19-20	53.10	98	4.36	3.78
18-18-19	52.00	1,106	4.40	3.88
17-17-18	45.96	1,210	4.64	3.83
16-16-17	45.41	667	5.91	4.25
15-15-16	44.77	425	5.07	4.33
14-14-15	42.43	308	4.31	3.58
13-13-14	42.86	226	5.06	3.75
12-12-13	43.71	113	4.60	3.61
12 and before	42.42	171	5.02	2.96

SUMMARY

In the longevity records of royalty, there is no significant correlation between age of parenthood and longevity of offspring.

A slight negative but significant correlation exists between order of birth and longevity of offspring when families of all sizes are grouped together. There are factors, however, which tend to bring about a spurious correlation of this kind if we unite data on families of different sizes.

When an attempt was made to avoid these sources of error by comparing the longevity of older and younger siblings there was found to be a slight tendency for the earlier-born brother to live the longer, but a similar tendency was not exhibited in the pairs of sisters.

Greater longevity of older brothers was significantly correlated with length of interval separating the births of older and younger brothers.

The correlations between fathers and children for length of life in royalty is $.1437 \pm .01126$, and between mothers and children $.1322 \pm .01355$.

A part of this correlation is probably spurious since it becomes considerably reduced when the material is broken up into groups extending through shorter intervals of time.

Parent-offspring correlations for longevity were reduced by the removal of deaths in the 0 to 4 age group.

Low mortality in early childhood is significantly associated with the attainment of advanced age by the parents.

The birth rate in royalty has been falling slowly since the sixteenth century but at an accelerated pace during the past fifty years.

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THE CIRCULATION OF BLOOD IN THE
LARVA OF *TRITURUS TOROSUS*

BY

J. FRANK DANIEL AND A. R. CURRLIN

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 16, pp. 377-386, plates 14 and 15, 4 figures in text

Issued September 29, 1928

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

THE CIRCULATION OF BLOOD IN THE LARVA OF *TRITURUS TOROSUS*

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INTRODUCTION

For the past few years the larva of the common newt, *Triturus (Diemyctylus) torosus* has been used in our laboratory to demonstrate the circulation of blood and to show some of the more significant features in this process. This paper is in part a summary of the observations made in our laboratory studies; in part it represents the continuous and detailed study of one of us (Currelin) on the larval circulation from the beginning of the heart beat to the 15-millimeter stage.

METHOD

The method employed consisted in anesthetizing the larva in a Stender dish containing tap water to which a few drops of chloretone had been added. A rectangular hole was cut in a piece of thin celluloid; the celluloid was then placed in the bottom of the Stender dish, and the larva was put in any desired position in the rectangular hole. The dish was then placed on a platform over a hole through which a beam of strong light could be reflected. The animal was observed mainly through dissecting binoculars at a magnification of 30 diameters. After the observations on the living specimen were concluded the larva was measured, killed, and fixed in Bouin's fluid, sectioned, and stained with Ehrlich's hematoxlin and erethrosin.

THE HEART AND ITS ACTIVITY

While the heart in *Triturus* begins its development at a stage when the body is from 3.5 to 4.0 mm. long it does not attain the form of an S-shaped tube until the body is from 5.5 to 6.0 mm. in length. It is at about the latter stage that the first heart beat takes place. At first the pulsation is a feeble wave-like contraction starting at the posterior end and traveling anteriorly. At these first pulsations, however, there is no flow of blood through the heart. Indeed, it is not

until the larva has reached another millimeter or millimeter and a half in length (7 to 7.5 mm.) that the vitello-intestinal or omphalo-mesenteric veins join the heart and the circulating fluid starts coursing through the heart tube. At first the contractions of the heart may be irregular but they soon settle down to a rhythmic action which persists throughout life.

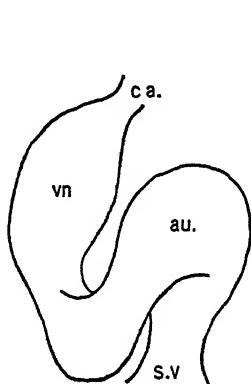


Fig. A

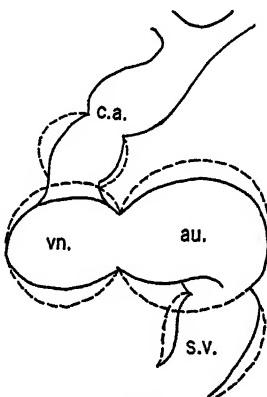


Fig. B

Fig. A. Heart in 8.5-millimeter stage of larva, *Triturus torosus*, ventral view. *au.*, auricle or atrium; *s.v.* sinus venosus; *t.a.*, truncus arteriosus; *vn.*, ventricle.

Fig. B. Heart in 15-millimeter stage of larva, *Triturus torosus*, showing systole (solid line) and diastole (dotted line), ventral view. Lettering as in figure A.

The mass which passes through the heart at first is milkish in appearance and without trace of coloring matter. In fact, haemoglobin is not present in the blood of *Triturus* until the 8 to 8.5-millimeter stage.

At 8.5 mm. the heart becomes constricted in various places giving to it a definite chambered appearance. These constrictions are found between the sinus venosus (*s.v.*, fig. A) and auricle (*au.*), between the auricle and the ventricle (*vn.*), and between the ventricle and the truncus arteriosus (*t.a.*). Figure B shows the rooms of the heart in systole (solid line) and diastole (broken line) in a still older larva (15 mm.).

From the 8-millimeter stage on the activity of the heart may be studied and accurately analyzed. Blood that is poured into the sinus venosus by the large veins is transported to the gills for oxidation. In this transportation the sinus venosus is the first to contract, carrying the mass of blood into the auricle, the ventricle, and out through the truncus arteriosus.

CIRCULATION IN THE GILLS

From the truncus the blood is forced forward through the short ventral aorta and the afferent arteries (*af.*, fig. C) to the gills. Its circulation in these channels, however, cannot be observed because of their depth, the density of the yoke in the younger specimens, and the density of the hyoid and branchial arches in the older.

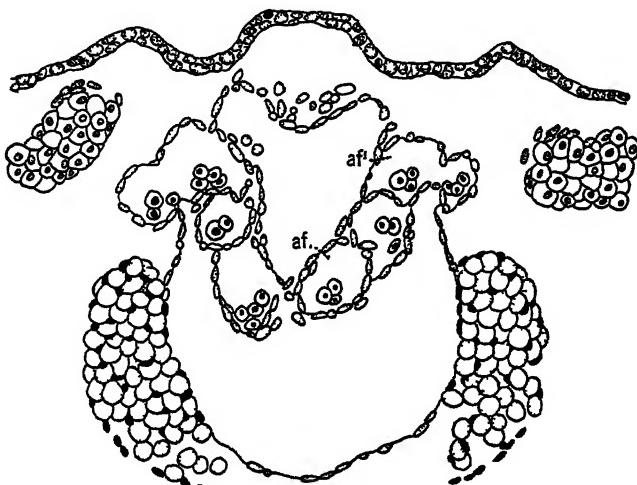


Fig. C. Cross-section showing afferent arteries (*af.*), as they leave the short ventral aorta, *Triturus torosus*.

In the gills the walls of the vessels are near the surface and the circulation can be observed to excellent advantage (pl. 14a). One gets the first view of this between the 7.9 and 8.5-millimeter stages, when circulation is established through the first aortic arch. In a brief time it is also completed in the second and third arches. At this stage there is one afferent (*af.*, pl. 14a) and one efferent artery (*ef.*) connected by numerous connectives or capillaries through the gill tissue.

Sections of the 15-millimeter stage show a small fourth aortic arch which does not enter an external gill. Its afferent arises from the proximal end of the third afferent and its efferent joins the efferent of the third arch. This arch gives rise to the pulmonary artery.

In a series of sections taken through these three different types of vessels it can be observed at this stage that all of them are alike in that the walls are essentially a single cell in thickness much like the capillaries of the adult (see Krogh, 1922).

Circulation in these vessels presents a rare picture. Here and there are eddies in which the corpuscles whirl rapidly around for a moment and then follow one another through the capillaries in stately procession. Since the walls of all the vessels in the gill area are devoid of a continuous muscular layer in the larval stage it is probable that all of them serve as membranes through which an exchange of gases is made.

CIRCULATION TO THE HEAD

In stages from 8.5 to 9 mm. in length branches of the internal carotid artery can be seen in the head area. The ophthalmic artery, supplying the cornea, lens, and certain other parts of the eye, enters this region from the posterior ventral part. The circulation can be seen to pass completely around the iris and to extend from the circle ray-like to deeper parts.

Another branch to the deeper part of the eye comes from the cerebral (internal carotid) artery, but this branch is not visible in surface view.

In the roof of the mouth a division of the internal carotid sends a branch outward which can be seen on the balancing organ. Circulation from the internal carotid at this point goes to three areas: (1) to the balancer, (2) to the operculum, and (3) to the maxilla. Blood from areas 2 and 3 passes mediad into the external jugular vein, while that from area 1 enters the anterior cardinal just back of the eye.

In an older stage (11.5 mm.) the external carotid artery is observed as a vessel which appears to rise from the first afferent artery. In a ventral view of the throat it can be seen running forward parallel with and laterad of the inferior jugular vein. During its course, the external carotid gives off the opercular artery and divides distally. The mediad division, the (posterior) mandibular artery, is drained through the (posterior) mandibular vein into the inferior jugular vein, while the lateral division joins the opercular vein which is also drained through the inferior jugular back to the heart.

The inferior jugular system of veins is not bilaterally symmetrical. The vein from one side takes its origin from two pairs of mandibular veins. Thus draining both sides of the mandible, it flows posteriorly in the midventral line. It soon inclines to one side or the other, receiving the opercular vein from that side, and reaches the heart

through the Duct of Cuvier. The vein of the other side is much shorter and smaller, taking its origin from the opercular vein at the place where the opercular vein from that side turns posteriorly.

CIRCULATION IN THE DORSAL AORTA AND ITS BRANCHES

The deeper, anterior part of the dorsal aorta is invisible in the living specimen but posteriorly in the region of the tail it is to be seen as the caudal aorta (see pl. 15).

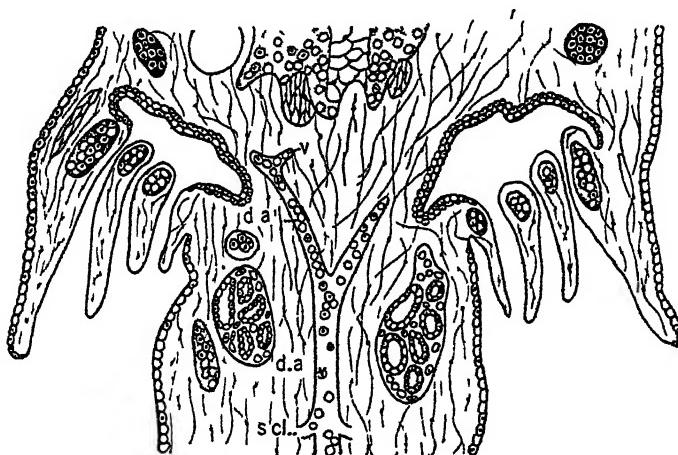


Fig. D. Frontal section through branchial area of 15-millimeter larva, *Triturus torosus*. *d.a.*, and *d.a'*, unpaired and paired dorsal aortae; *s.cl.*, subclavian artery; *v.*, vertebral artery.

In section (fig. D), two of the anterior branches of the dorsal aorta are shown. One of these is the vertebral (*v.*); the other is the important subclavian artery (*s.cl.*).

CIRCULATION IN THE FOREARM

In the 11.5-millimeter stage the subclavian, as the brachial artery, extends down the central axis of the forearm (pl. 14b). In the foot this vessel divides, supplying a branch to each of the digits. From the digits the currents are seen to turn backward in two veins which course along the ulnar and radial sides of the arm, respectively. These two vessels are seen in sections to join and then to enter the postcardinal system.

A branch, the hypogastric artery, is given off of the subclavian just before it enters the fore limb. This vessel runs medially and posteriorly along the abdominal wall near the midventral line and is in all essential respects like the lateral abdominal artery of elasmobranch fishes (Daniel, 1928).

CIRCULATION ON THE VISCERA

The unpaired branches leaving the dorsal aorta between the subclavian to the fore leg and the iliac to the hind leg go to the viscera. Circulation in most of these, however, can be seen only where the arteries leave the dorsal aorta; in the deeper parts of the vessels on the viscera it can rarely be followed. This blood passes back to the heart by the hepatic portal system of veins.

At about the 9.5-millimeter stage the downgrowing liver diverticulum divides the omphalomesenteric vein into two parts. The anterior segment becomes the hepatic vein draining blood from the liver into the sinus venosus, while the posterior segment, the subintestinal vein, empties its blood from the yolk sac also into the liver. As the intestine becomes coiled the subintestinal vein changes its course, running deep into the abdomen just behind the first coil of the duodenum. Here it is joined by a supraintestinal vein, forming a hepatic portal vein which drains into the liver.

CIRCULATION IN THE TAIL

The caudal aorta (*c.a.*, pl. 15) sends segmental branches (*sg.*) both dorsally and ventrally. Those dorsally, supply the dorsal lobe of the caudal fin; while the ventral branches provide for the ventral lobe. At the area of the cloaca the first and second dorsal segmentals and the first ventral segmental lead dorsally and anteriorly into the lateral cutaneous vein (*l.c.v.*), which carries the blood forward to the postcardinal sinus, from which it reaches the sinus venosus. The remaining dorsal and ventral segmentals enter the caudal vein (*c.v.*). At the area of the cloaca the caudal vein divides into the right and left renal portal veins (*rn.*). Blood is carried anteriorly by these along the mesonephrotic kidney and finally also reaches the postcardinal sinus.

In no part of the animal does the circulation present a more remarkable picture than in this area.

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EXPLANATION OF PLATES

PLATE 14

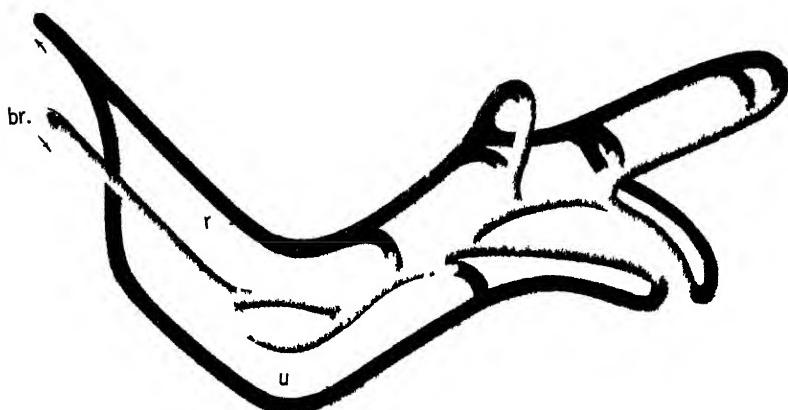
a. Circulation in external gill, *Triturus torosus*. Arrows denote direction of current. (Paul Burlingame, orig.)

b. Circulation in forearm, 15-millimeter larve, *Triturus torosus*. Arteries in red; veins in blue. (Paul Burlingame, orig.)

af., afferent artery (blue); *br.*, brachial artery; *cp.*, connectives or capillaries; *ef.*, efferent artery (red); *r.*, vein on radial side; *u.*, vein on ulnar side.



a



b

PLATE 15

Circulation in tail of 15-millimeter larva, *Triturus torosus*. Arteries in red; veins in blue. (Harold Lew, orig.)

c.a., caudal artery; *c.v.*, caudal vein; *d.a.*, dorsal aorta; *l.c.*, lateral cutaneous vein; *rn.*, renal portal vein; *sg.*, segmental artery.

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[DANIEL CURRLIN] PLATE 15



THE EFFECTS OF RADIUM AND RADIUM
IN COMBINATION WITH METALLIC
SENSITIZERS ON ENDAMOEBA
DYSENTERIAE IN VITRO

BY

ELIZABETH CUSTER NASSET AND CHARLES A. KOFOID

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 17, pp. 387-416, plates 16, 17, 7 figures in text

Issued November 8, 1928

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

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INTRODUCTORY AND HISTORICAL

The primitive peoples believed light and heat to be the source of all life. The name of the Egyptian sun god, Ra, persists today in our word *radiation*. The effects of light and heat on plant and animal life and upon non-living substances have inspired man with fear, wonderment, and reverence since the beginning of his existence. Modern mechanism has replaced the idea of mystery associated with the action of light, yet, even today, we are still unable to give a true picture of what actually takes place when plant and animal cells are acted upon by radiation.

Radiation was first used therapeutically by Löbel (1815), who attributed its beneficial action to some chemical power of the rays. Charcot (1858), in the study of erythema of the eyes produced by radiations from an electric arc, decided that it was brought about through some chemical action of the light. Downes and Blunt (1879) suggested that the destructive action of light was the result of the oxidizing power of the rays, while later workers have ascribed its toxic effects to the various products of its activity.

Schwarz (1903) believed that the toxic action was due to the splitting off of lecithins, and Schaper (1904), that it was due to the products of the split lecithins. Hertel (1905) thought that oxygen was split off from the components of the protoplasm. Hertwig (1911) was of the opinion that radiations act upon nuclear components, probably chromatin, producing what he termed a "living toxine."

According to Neuberg (1904) the effect of radiation on carcinoma tissue is to destroy all enzymes except the autolytic. Evidence regarding the action of radiation on enzymes is most contradictory. Some workers have found that the activity of enzymes is increased after irradiation, others have found no effect to be produced, while some claim that the result of their activity is destruction.

A great variety of the lower plants and animals, and the tissues of higher animals have been exposed to the action of radium rays. It is not our purpose to review the vast amount of literature on this subject. Pacinotti and Porelli (1899) were the first to expose pathogenic organisms to the action of a radio-active substance. The organisms of cholera, tuberculosis, diphtheria, and typhoid were exposed to preparations of uranium powder with lethal effects. Subsequent workers have not been able to duplicate their results.

A number of free-living and parasitic Protozoa have been subjected to radium radiations. Willcock (1904) found that different Protozoa varied greatly in their response to radiation. An organism containing chlorophyll, such as *Euglena*, was found to be remarkably resistant.

Bovie and Hughes (1918) state that quartz ultra-violet decreases the division rate in *Paramecium caudatum*, the effect increasing with increased length of exposure. Short exposures may produce brief inhibition followed by acceleration. The effect of quartz ultra-violet is on the nucleus, while fluorite ultra-violet, with a difference in wave length of $.12\mu$, produces cytolysis. . .

Bruynoghe and Dubois (1925) found that radium affects the virulence for mice but not the motility of *Trypanosoma gambiense* and *Trypanosoma brucei ugandae*.

The greater part of the earlier work was not well controlled and was without a quantitative basis. Every effort is made today to control experiments accurately and to place them as nearly as possible on a mathematical foundation.

Strangeways and Hopwood (1926) determined the effects of X-rays upon mitotic cell divisions in tissue cultures *in vitro*. Canti and Donaldson (1926) performed similar experiments with radium. In both cases, actual counts of the cells in mitosis were made after a given exposure to radiation. The authors state that in none of these experiments was there any evidence of a stimulating effect on cell division due to radiation.

Crowther (1926) studied the effects of X-radiation on *Colpidium colpoda*. He found that 75 per cent of the lethal dose caused stimulation, organisms two to two and one-half times larger than the controls being produced.

Among the parasitic Protozoa, *Opalina*, *Nyctotherus*, *Balantidium*, *Trypanosoma*, and certain Sporozoa have been subjected to radiation with varying results. The majority of workers report only slight effects due to radiation. So far as we know, no work has been done outside of this laboratory on the effects produced by radiating parasitic amoebae of the human intestine. We believed that it would be useful to study *Endamoeba dysenteriae* as subjected to radium rays *in vitro*, with the ultimate view of suggesting, if possible, a therapeutic use for radium in the treatment of human amoebiasis.

ACKNOWLEDGMENTS

Sincerest thanks are given to Professor Sumner C. Brooks, who made many helpful and valuable suggestions in the course of this research and who, in particular, furnished information upon the physics of radiation. Acknowledgment is made to Dr. Emil G. Beck who supplied the radium capsule and needles and has maintained a deep interest in the work.

MATERIAL AND METHODS

The strain of *Endamoeba dysenteriae* used in these experiments was isolated from a chronic case of dysentery four months previous to the beginning of this work. This was the youngest strain of *E. dysenteriae* under cultivation at this time. It was thought best to use a recently isolated strain as such a strain is generally more virulent and less resistant than one which has long been under cultivation.

Stock cultures of *E. dysenteriae* were grown in Locke's egg blood (L.E.B.) medium according to the method of Kofoid and Wagener (1925). The amoebae grow on a solid base of coagulated egg in Locke's solution containing 0.5 per cent defibrinated rabbit's blood.

The egg slants are made of whole egg diluted with Locke's. Attempts were made to grow the amoebae in a more simple medium, in order to do away with any filtering of the radiations by the proteins of the stock medium. While the organisms lived in Locke's solution alone, all cultures would not survive transplanting. The usual medium was used then, as growth in it is always dependable. Preliminary tests were made with various dilutions of the salts employed to determine the dilution that the amoebae would tolerate and in which they would bear transplanting indefinitely.

The radium used in these experiments was in three forms; platinum needles loaned by Dr. E. G. Beck, of Chicago, containing $12\frac{1}{2}$ mgm. of radium in the form of radium sulfate and furnishing some beta and all of the gamma radiations; $12\frac{1}{2}$ mgm. sealed in a brass capsule; and a radium plaque containing 10.32 mgm. loaned by the University of California School of Medicine.

The experiments employing needles were carried on in small culture tubes with an inside diameter of 1 cm. The total volume of culture was 4 cc. in all experiments. Inoculations approximated as nearly as possible 10,000 organisms. The inoculum was taken from a 24-hour culture which had been multiplied by daily transplants. Two or three such cultures were concentrated and the number of amoebae in 1 cc. accurately counted in a blood-counting chamber which serves admirably for this purpose. To insure a uniform mixture of amoebae, the inoculum was mixed 40 times with a pipette before counting and before inoculation.

The radium needles or capsule, sterilized by boiling for one-half hour, were lowered by threads in the culture tube (fig. 1). The point of the needle or capsule rested at the base of the egg slant in almost immediate contact with the amoebae. When the radium plaque was used it was necessary to employ a slightly different technique. Vials with an inside diameter of about 2 cm. were prepared. The plaque, held in a sterile rubber cot, was placed, radium



Fig. 1

side downward, into the medium close to the egg slant and in a position as nearly parallel to the slant as possible. The culture tubes, containing the radium were wrapped in a sheet of lead 1 mm. thick and placed in a lead chamber in a water bath regulated to 37.5° C. Control tubes were placed in an incubator on the opposite side of the room.

Immediately after the required exposure, counts of the exposed culture and the control were made with the blood-counting chamber. Two culture slides from the exposed tube and one from each control were fixed in hot Schaudinn's fluid at the close of each experiment and subsequently stained with iron haematoxylin. Transplants of the exposed cultures and of controls containing the sensitizing salt were made into culture tubes and maintained for several weeks to determine the after-effects of the radiation. Slides were prepared, especially during the first few days after irradiation, in order to study the duration of the morphological effects produced.

All experiments were controlled and checked. Two controls, generally, were prepared and averaged.

EXPERIMENTAL

Effects of radium alone—

Experiment 1.—An inoculum of 10,000 amoebae was exposed for 12 hours to 12½ mgm. of radium in a brass capsule. Definite stimulation occurred. The number of amoebae in the exposed tube approximated three times that of the control. While the size of the amoebae was not appreciably increased, stained slides revealed interesting nuclear changes. In several forms the peripheral chromatin was pulled away from the nuclear wall and lay as an irregular thread within the nucleus (pl. 16, fig. 5). In some amoebae the central karyosome stained faintly. There were slight variations in the size of the nuclei. Both large and small ones showed signs of disintegration or exhibited abnormal mitotic figures. In the larger amoebae the cytoplasm was often considerably vacuolated. The greater number of organisms on the slide, however, appeared quite normal.

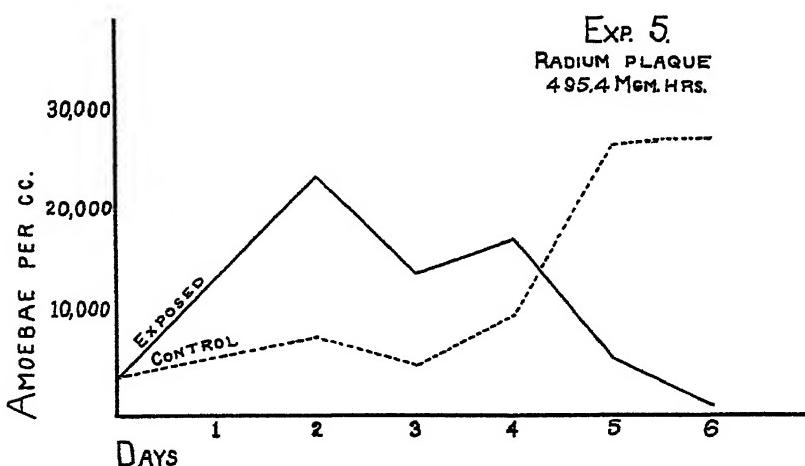
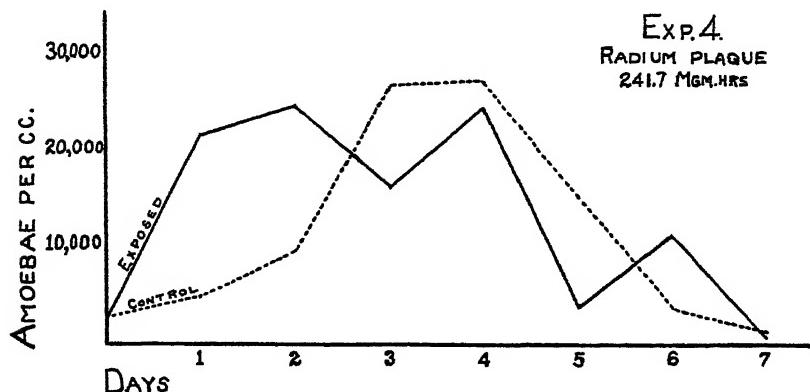
Experiment 2.—An inoculum of 10,500 amoebae was exposed for 24 hours to 12½ mgm. of radium in a capsule. Stimulation occurred as in experiment 1, with over three times as many amoebae being produced in the irradiated culture as in the control. Some increase in size over those of the control was observed. The exposed amoebae

were large, active, and hyaline. The control exhibited a larger number of rounded-up organisms than the exposed tube. Nuclear changes were similar to those observed in experiment 1, but in some cases more pronounced. The chromatin was pulled away from the nuclear wall in a comparatively large number of amoebae. Exposure to $12\frac{1}{2}$ mgm. of radium in a needle gave similar results.

Experiment 3.—The most marked morphological variations were noted when an inoculum of 9000 amoebae was subjected to the radiations from $37\frac{1}{2}$ mgm. of radium for 12 hours. The exposed tube contained nearly three times as many organisms as the control. Huge, very active forms, as well as rounded-up, or sluggish granular forms were present. There were bits of protoplasm observed about the size of, or smaller than, the normal control (pl. 16, figs. 1, 2). Plate 16, figure 4 shows an enucleated bit of protoplasm about the size of a normal amoeba. In a large number the cytoplasm increased from two to over three times as much as in the control, accompanied by a corresponding increase in the nuclear diameter. There was a decided absence of definite pseudopod formation in these forms. The most conspicuous nuclear changes were a tearing away of the peripheral chromatin from the nuclear wall (pl. 17, fig. 13); the massing of this chromatin (pl. 16, figs. 6, 7) sometimes producing forms grossly resembling *Councilmania dissimilis* Kofoed; structureless karyosomes staining faintly; and general disintegration of the chromatin (pl. 17, fig. 14). One large form (pl. 17, fig. 13) was observed containing two nuclei each of which exhibited the effects of radiation. Nuclei varied greatly in their staining qualities. Some were very faint and others were so deep that structures could only be made out with difficulty. The irradiated amoebae from the above experiments were maintained in culture for three months and were then given up. The effects of radiation both upon growth rate and upon morphology disappeared in five to eight days.

Experiment 4.—An inoculum of 12,000 amoebae was exposed to the 10 mgm. radium plaque unscreened, except for the rubber cot, for 24 hours. Control and irradiated cultures were counted at the end of the exposure. An inoculum of 10,000 amoebae from each tube was transplanted into culture tubes and after 24 hours' incubation, counts made as before. This procedure was repeated until the amoebae decreased so in number that accurate counts could not be made. The results of this experiment are represented graphically in figure 2. At the end of 24 hours' irradiation there were over four

times as many organisms in the exposed tube as in the control. After 48 hours the count in the exposed tube had risen along with the control, but to a less extent. On the third day the irradiated culture fell, in contrast to a decided rise in the control. This was followed by a secondary rise on the fourth day, the control still being main-



tained at its previous high level. Both irradiated cultures and controls fell off on the following day and on the seventh day counts were very low in both tubes. The control culture was the first to rise.

The size of the radiated amoebae did not increase to any considerable extent over the controls. The majority exhibited a normal nuclear picture. In a few, the peripheral chromatin had commenced to form a blob on one side (pl. 16, fig. 3) but in no case were the

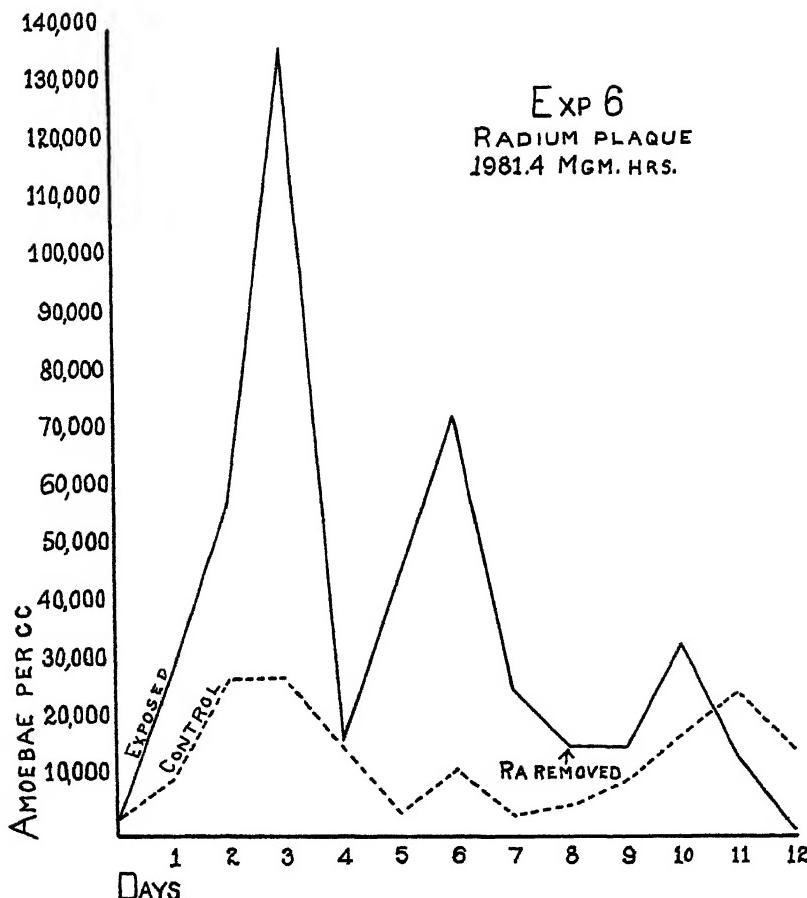


Fig. 4

changes so extensive as in the previous experiments. The karyosome appeared normal. In some, it stained even more deeply than the controls.

Experiment 5.—In this experiment an inoculum of 14,000 amoebae was exposed to the 10-mgm. plaque for 48 hours. Daily transplants and counts were made as in experiment 4. The results are represented in figure 3. After 48 hours' irradiation, there were approximately three times as many amoebae as in the control. The count was reduced on the third day along with a reduction in the control. The secondary rise was observed on the fourth day as in experiment 4, followed by a decided drop in contrast to a definite rise in the controls. No striking differences in morphology were noted.

TABLE 1
10-MGM. PLAQUE 8 DAYS, 1,981.5 MGM. HOURS

Experiment	Inoculum	Tube	Exposure, hours	Mgm hours	Amoebae per cc
6a	10,200	1	24	241 2	28,800
		2			10,000
6b	10,000 from 1a	1	24	241 2	57,200
	10,000 from 2a	2			27,200
6c	10,000 from 1b	1	24	241.2	136,250
	10,000 from 2b	2			27,800
6d	10,000 from 1c	1	24	241 2	16,700
	10,000 from 2c	2			15,700
6e	10,000 from 1d	1	24	241 2	44,700
	10,000 from 2d	2			4,000
6f	10,000 from 1e	1	24	241.2	72,500
	10,000 from 2e	2			11,500
6g	10,000 from 1f	1	24	241 2	25,300
	10,000 from 2f	2			3,800
6h	10,000 from 1g	1	24	241 2	15,300
	10,000 from 2g	2			5,300
6i	10,000 from 1h	1			15,300
	10,000 from 2h	2			10,000
6j	10,000 from 1i	1			33,750
	10,000 from 2i	2			18,000
6k	10,000 from 1j	1			13,750
	10,000 from 2j	2			25,000
6l	10,000 from 1k	1			0 3 amoebae on slide
	10,000 from 2k	2			15,000

Experiment 6.—Very interesting results were obtained when *Endamoeba dysenteriae* was exposed continuously for eight days to the unscreened radiations from the 10-mgm. plaque. After 24 hours' exposure, counts and inoculums of 10,000 amoebae were made as above, and the radium placed in again. The highest stimulation occurred on the third day, 136,000 amoebae being present in the exposed tube. The highest stimulation in comparison with the control occurred on the sixth day—72,000 in the exposed tube compared with 11,000 in the control. The results are expressed in table 1 and, graphically, in figure 4. Definite nuclear changes were not observed

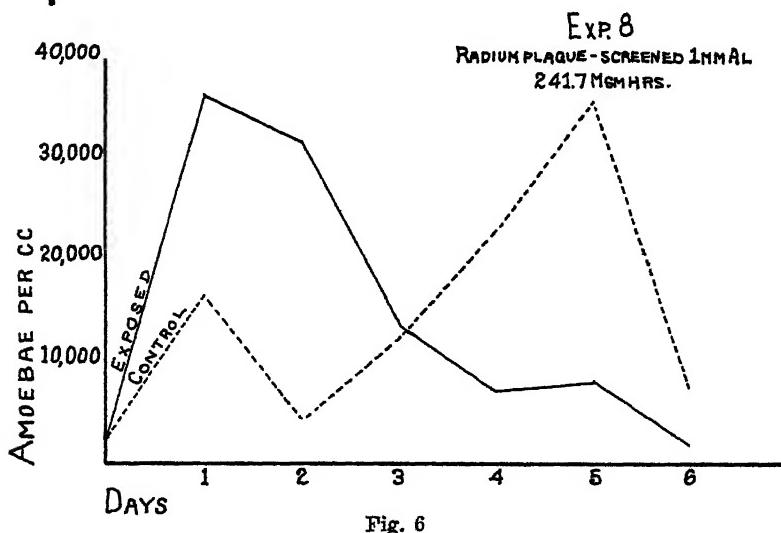
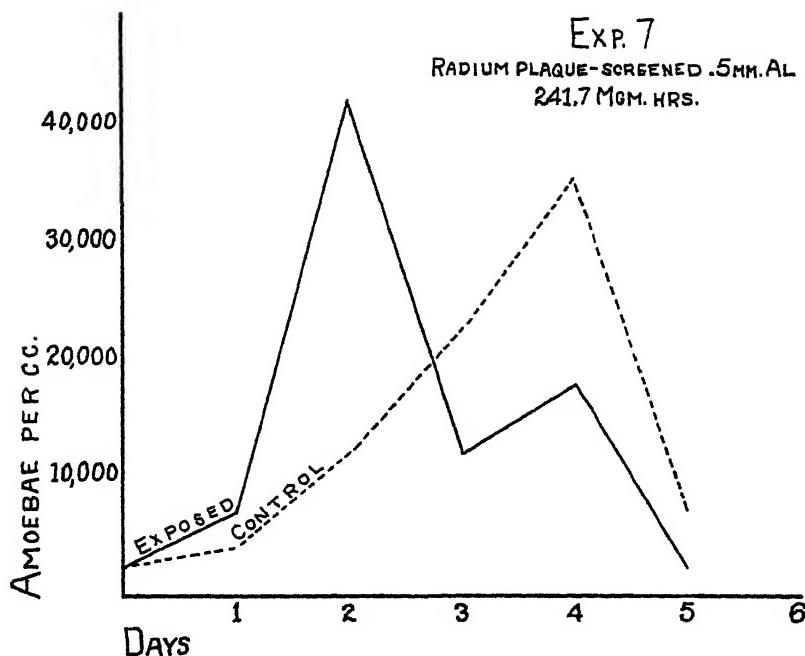
TABLE 2
IONIZATION PRODUCED BY 10-MGM. PLAQUE

Screening	Fall in scale divisions per second
1 None	16 5
2 0.5 mm. Al	6 66
3 1.0 mm. Al	2 9
4 1.5 mm. Al	1 8

until the third day. Changes similar to those found in experiment 3 were noted with some interesting variations. The amount of chromatin was conspicuously reduced in a few amoebae. In others the chromatin was irregular or broken (pl. 17, fig. 9). A small number of nuclei stained deeply and resembled somewhat the clouded nuclei observed in the lymph glands of Hodgkin's disease (Kofoid, Boyers, and Swezy, 1922). In a few nuclei the karyosome appeared quite eccentric. Some nuclei were found in the outermost boundary of the cytoplasm in the region of the ectoplasm. They seemed to be on the verge of being extruded. No enucleated bits of protoplasm were observed, however. In many cases the cytoplasm was irregular and ragged in outline.

Experiment 7.—A study of the effects produced by screening out the alpha and various proportions of the beta rays was made by using the 10-mgm. plaque in combination with aluminum screens. Preliminary to running the experiments, the ionization produced in the air by the plaque with various amounts of screening was measured by means of a gold-leaf electroscope. The results are expressed in table 2.

An inoculum of 10,000 amoebae was exposed for 24 hours to the radiations from the plaque screened with 0.5 mm. of aluminum. The



results are represented graphically in figure 5. The slight initial stimulation is the most noticeable feature of the graph. The exposed tube contained less than two times as many amoebae as the control, compared with a stimulation of three to four times in the unscreened exposures. Upon repetition of this experiment a stimulation of twice that of the control was obtained. On the second day the count rose sharply in comparison with a less significant rise in the control.

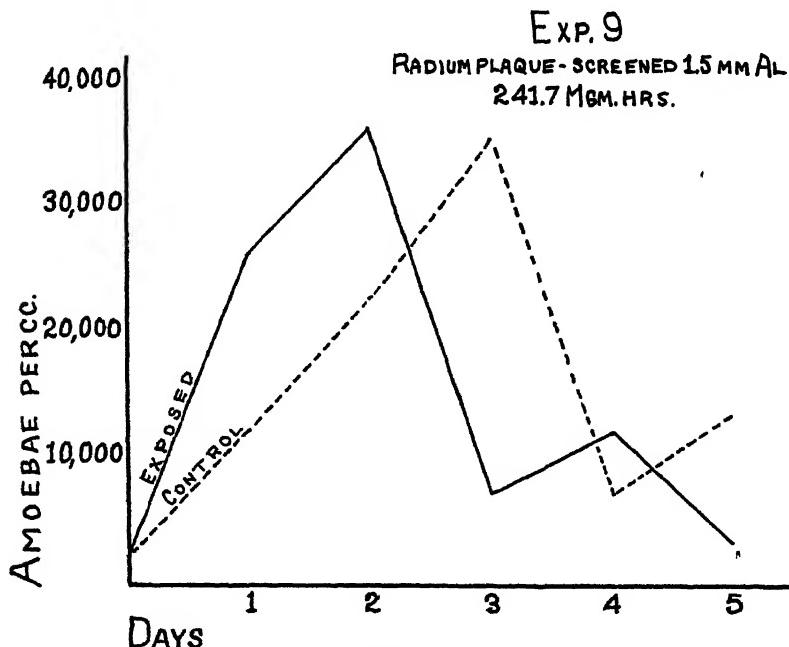


Fig. 7

Experiment 8.—One mm. of aluminum screening was employed and the amoebae radiated as above. Figure 6 gives the results. An initial stimulation producing over two times as many as the control will be noted. On the second day the count fell along with the control.

Experiment 9.—Screening was tried with 1.5 mm. of aluminum and the amoebae irradiated as in experiment 7. The results (fig. 7) indicate that definite stimulation occurred. On the second day the exposed culture rose along with the control, but fell sharply on the third day, the control continuing to rise.

Experiment 10.—The effects of screening with 5 mm. of aluminum were studied and results similar to the above were obtained.

In the above series of experiments, in which screening was employed, it will be noted that in no instance did the exposed cultures

fall so low as in the experiments in which the unscreened plaque was used. Nuclear changes similar to those observed with the unscreened radium were often obtained. While the karyosome generally appeared normal, in a considerable number the peripheral chromatin was torn away from the nuclear wall.

Effects of radium and sensitizer—

Experiment 11.—*Endamoeba dysenteriae* in cultures containing ferric chloride in dilutions of 1-1,000,000, 1-100,000, and 1-50,000 was exposed for 12 hours to 12½ mgm. of radium in a brass capsule. FeCl₃ in combination with radium did not produce a lethal effect. The division rate increased as in tubes containing no iron salt with the production of two to three times as many organisms as in the control.

Experiment 12.—The amoebae were exposed to strontium chloride in a dilution of 1-50,000 plus 12½ mgm. of radium for 12 hours. Stimulation occurred as above.

Experiment 13.—Potassium iodide in various dilutions was employed in combination with 12½ mgm. of radium. While no lethal effect was produced by the use of KI and radium together, the marked stimulation which took place in the experiments in which iron or strontium were used, did not occur. Exposed tubes averaged only slightly higher than controls.

Experiment 14.—Gold chloride in dilutions of 1-1,000,000, 1-100,000, and 1-50,000 and 12½ mgm. of radium for 12 hours failed to produce any lethal effects. The amoebae were stimulated by the radium as in tubes which did not contain gold.

Experiment 15.—The results of using various dilutions of mercuric chloride in combination with radium are expressed in table 3. The lethal effect of radium in combination with mercury seems to depend upon three factors: (1) the concentration of mercuric salt used; (2) the amount of radium; and (3) the length of exposure. A 1-50,000 dilution of HgCl₂ was found to be the most effective. A 1-50,000 dilution, in combination with 12½ mgm. of radium for 12 hours, produced an almost completely lethal effect. The same dilution and amount of radium as above for 24 hours definitely killed all amoebae; 37½ mgm. of radium for 12½ hours in a 1-50,000 dilution of HgCl₂ also produced definitely lethal effects.

Figure 11, plate 17 is taken from a culture which contained HgCl₂ in a 1-100,000 dilution and was irradiated with 12½ mgm. for 12

TABLE 3
HgCl₂ AS SENSITIZER

Experiment	Inoculum	Tube	Exposure hours	Mgm hours	Dilution of sensitizer	Transplants	Amoebae
15a	8,000	1	12 capsule	150 0	1-1,000,000	Negative after second transplant	4,300
		2			1-1,000,000	Survived	11,500
		3					13,000
15b	9,000	1	11½ capsule	143 8	1-100,000	Negative after third transplant	3,000
		2			1-100,000	Survived	15,600
		3					14,200
15c	11,000	1	12¼ capsule	153 0	1-50,000	Negative after first transplant	1 motile amoeba
		2			1-50,000	Survived	10,300
		3					12,800
15d	10,000	1	24 capsule	300 0	1-50,000	Negative	0
		2			1-50,000	Survived	21,300
		3					18,900
15e	10,500	1	12 needles	350 0	1-50,000	Negative	0
		2			1-50,000	Survived	17,400
		3					18,500

hours. The chromatin is seen to be affected as in other irradiated cultures and the size of the amoeba is increased somewhat over the controls. The majority of organisms, however, appeared normal. HgCl₂ in the dilutions used caused hemolysis of the red cells of the medium, but this was without effect on the normal growth of the amoebae in the control.

Experiment 16.—The action of lead in combination with radium is of a similar order to that of mercury. Mercury produces somewhat more completely lethal effects, as will be seen from the results

TABLE 4
 PbCl_2 AS SENSITIZER

Experiment	Inoculum	Tube	Exposure hours	Mgm hours	Dilution of sensitizer	Transplants	Amoebae
16a	11,000	1	11 $\frac{3}{4}$ capsule	146.9	1-1,000,000	Negative	1,500
		2			1-1,000,000	Survived	20,400
		3					18,200
16b	9,500	1	12.5 capsule	156.3	1-100,000	Negative after second transplant	4,700
		2			1-100,000	Survived	16,300
		3					14,900
16c	10,000	1	12 capsule	150	1-50,000	Negative after fourth transplant	8,600
		2			1-50,000	Survived	12,300
		3					14,100
16d	9,000	1	24 capsule	300	1-50,000	Negative	0
		2			1-50,000	Survived	15,300
		3					14,600
16e	9,500	1	12 needles	350	1-50,000	Negative after third transplant	2,800
		2			1-50,000	Survived	22,600
		3					24,800

in table 4. The stained slides revealed an unexpected picture. Nearly every amoeba appeared perfectly normal. Some were found containing ingested red cells. Figure 10, plate 17, is taken from a culture which contained PbCl_2 , 1-50,000 and was irradiated for 12 hours with $37\frac{1}{2}$ mgm. Every nucleus on the slide appeared normal and the majority of them were in mitosis.

Irradiation of the medium containing the lead or mercury salt for 12 or 24 hours previous to the inoculation did not yield any lethal effects.

The experiments with lead and mercury salts were repeated using the 10-mgm. plaque as the source of radiation. These experiments were performed with the same strain of amoebae as used previously but about two months after the completion of the first group of experiments in which metal salts were employed. It was soon evident that the amoebae had become more sensitive to mercury. They were no longer able to withstand a dilution of 1-50,000 and sometimes did not grow well in a 1-100,000 dilution. A 1-200,000 solution was used.

TABLE 5
10-MGM. PLAQUE AND $HgCl_2$

Inoculum	Tube	Exposure hours	Mgm. hours	Dilution of sensitizer	Transplants	Amoebae
12,800	1	24	241.2	1-50,000	Survived	12,200
	2	1-50,000	Negative	0
	3	16,900

The amoebae remained capable of withstanding a 1-50,000 dilution of lead chloride. In all of these experiments in which a sub-lethal dilution of the metal salt was employed, definite stimulation occurred as a result of radiation. The amoebae increased to two or three times as many as in the controls. In one experiment in which a 1-50,000 dilution of $HgCl_2$ was used, stimulation took place in the exposed tube in spite of the death of all of the amoebae in the control containing mercury (table 5).

It was suggested that the contradictory results might be due to the beta rays which were not screened out in these experiments to the extent that they were when needles or capsules were used. The plaque was screened with 1 mm. of aluminum which cuts out 99 per cent of the beta rays. Stimulation occurred as before. The use of 1.5 mm. of aluminum as a screen did not alter the results to any considerable extent.

Each type of experiment was checked with our strain 3, which has been maintained in culture for about three years, and similar results were obtained.

DISCUSSION

It is difficult to harmonize the results of these experiments with some of the findings of other observers. The majority of investigators state that radium rays, in amounts comparable to those used in these experiments, do not cause increased division rate of cells *in vitro*. In all our experiments in which radium alone was employed, definite stimulation occurred, even with large amounts ($37\frac{1}{2}$ mgm. for 12 hrs.). Miss Allen, in this laboratory, exposed *Councilmania dissimilis* to 85 mgm. of radium for 24 hours and then to $122\frac{1}{2}$ mgm. for 48 hours, with decided stimulation taking place. This culture survived transplantation. *Endamoeba dysenteriae* resembles *C. dissimilis* in its cultural requirements and in its susceptibility to various disinfectants.

Lazarus-Barlow and Beckton (1913) using minute quantities of radium (5×10^{-7} mgm. for 30 hrs.) on the ova of *Ascaris megalcephala* found that stimulation in division rate occurred. Larger quantities than the above, or more prolonged exposures, progressively retarded the rate of division. According to Bovie and Hughes (1918) who studied the effects of quartz ultra-violet on the division rate of *Paramecium caudatum*, only one-thirtieth of the amount of radiation necessary to produce cytolysis is required for inhibition. If rays penetrate into a certain portion of the cell, some physiological reaction takes place regardless of the wave length (Bovie, 1918a). Malformed embryos are produced when *Ascaris* eggs are radiated with ultra-violet of long wave length. Similar results are obtained when frog's eggs are exposed to radium rays. These results with radiations of decidedly different wave lengths tend to indicate that the physiological mechanism concerned seems to play a greater part, in determining the effect, than the wave length of radiation employed. From this rather loose analogy, we might conclude that the dosage of radium required to produce inhibition in *Endamoeba dysenteriae* would have to be considerably larger than any employed in our experiments, and that the amount required to produce a lethal effect would have to be thirty times the inhibitory dosage. This would call for the use of comparatively enormous quantities of radium to produce any fatal effects.

Bovie and Hughes (1918) state that short periods of inhibition may be followed by acceleration of cell division in *Paramecium caudatum* exposed to quartz ultra-violet.. We have found in our experiments that stimulation in division rate is the initial effect of radiation. For the first day after irradiation, exposed and control cultures behave similarly. Both rise together (figs. 2, 5, 7) and fall together (figs. 3, 6). The most prominent after effect of radiation is a fall in the exposed culture accompanied by either a rise or fall in the control depending upon its own cycle. *Endamoeba dysenteriae* exhibits a more or less constant growth cycle in culture, as may be observed by examination of the control curves. High peaks of division rate occur approximately every six days with usually smaller peaks in between. This cycle is not absolutely fixed, as it is dependent upon a variety of factors, particularly the cycles of the bacteria accompanying the amoebae in culture. All of these experiments are meaningless unless rigid comparisons are made with the controls. The tendency, then, is for the irradiated culture to follow its own growth curve parallel with the control for the first day after the cessation of radiation. On the third day, however, this is overcome by the after effects of the radiation and the exposed culture often falls off in comparison with the normal rise in the control. A secondary rise is frequently observed in the exposed tube followed by a decided reduction in division rate. In no case, however, did a radiated culture die out after successive transplants. In experiment 8 (fig. 4) in which the amoebae were exposed continuously for eight days to 10 mgm. of unscreened radium, it will be noted that the exposed culture in a general way followed the control cycle, and did not markedly fall off until the radium was removed. It is interesting to note that the tremendous stimulation culminating on the third day was followed by just as decided a decrease in division rate. An inoculum of 2500 amoebae per cc. producing 136,000 at the end of 24 hours means that every amoeba divided on the average of a little over every four hours, which indicates a very high metabolic rate. *E. dysenteriae* grown in Ringer's egg starch medium according to the method of Dobell and Laidlow (1926) is stimulated to enormous growth both in size and number of organisms. This growth seems to be directly dependent upon the amount of food present and is not comparable to the stimulation produced by radiation.

Bruynoghe and Mund (1925) state that gamma rays are without action on bacteria, but that alpha and beta rays are distinctly bac-

tericidal. Chambers and Russ (1912) also had arrived at a similar conclusion. Wedd and Russ (1912) and Mottram (1926) ascribe to beta radiation the inhibitory effect on further growth of irradiated mouse carcinoma or Jensen's rat sarcoma inoculated into new experimental animals. Canti and Spear (1927) state that gamma rays fail to produce any stimulating action on tissue cells in culture. We have found that *Endamoeba dysenteriae* exposed to 10 mgm. of unscreened radium for as long as eight days is not killed and can be maintained in successive cultures. Screening does not remove the stimulative effects of radiation, although division rate as high as with unscreened radium has not been obtained. The after effect, or reduction in division rate, was not so prominent when screened radium was employed and therefore it seems reasonable that beta rays may play some part in producing this effect.

One very significant result of radiation was noted in cultures which had been exposed to both screened and unscreened radium. Such cultures were "cleaned up." The bacterial flora was markedly reduced in many cases. If such cultures had not survived transplantation, we would be at a loss to know whether to ascribe their death to the direct action of the radiations on the amoebae or to the elimination of bacteria necessary for their growth. The remarkable resistance of *E. dysenteriae* to radium rays suggests a means of cleaning up cultures in an attempt to grow the organisms in a pure state.

It is interesting to note here, in view of the work done by Kofoid, Swezy, and Boyers (1922) on the coexistence of Hodgkin's disease and amoebiasis, and the finding of *E. dysenteriae* in the lymph glands of man in Hodgkin's disease (Kofoid, Boyers, and Swezy, 1922), that Hodgkin's disease is extremely radiosensitive (Beck, 1924). In the light of the experiments on *E. dysenteriae* exposed to radium rays *in vitro*, we would conclude that this organism is very radio-resistant. We do not know of any data on the effects of radiation on the amoebae in the lymph glands of Hodgkin's disease. This would prove an interesting problem. Here we are dealing with a different situation. *E. dysenteriae*, *in vivo*, may prove to be very susceptible to radiation.

Haendly (1918) has carefully studied the changes produced in rayed carcinoma cells. He states that the nuclei become larger in proportion to the cell bodies, although some nuclei get smaller and smaller and finally disappear. We did not find any striking increase in the size of the nuclei over that of the cell body in irradiated amoebae, nor any reduction in size of nuclei. But we did find the

amount of chromatin to be reduced, or a massing or disintegration of this chromatin. The most marked nuclear changes were produced when a large amount of radium acted for a short time rather than when a smaller amount acted for a longer time (see pl. 16, figs. 6-8, and pl. 17, figs. 13, 14 in comparison with pl. 17, fig. 9). The enucleated bits of protoplasm found after irradiation with $37\frac{1}{2}$ mgm. may have been due to extrusion of the nucleus or to autotomy. They were not observed on the control slides.

The lethal effect of radium in combination with mercury and lead salts may be due to one of four factors or to a combination of any of these four:

1. Direct effect of radium rays.
2. Direct formation of a toxic valence.
3. Secondary radiation or collision of the first type.
4. Radiationless transfer or collision of the second type.

The direct effect of radium rays in causing death is ruled out. We have seen above that stimulation in division rate always occurs when *E. dysenteriae* is exposed to radium alone and that in no case did death result as an after effect.

It is possible that mercuric mercury under the influence of radium may be reduced to the mercurous state, which may be more toxic for *Endamoeba*. Pougnet (1915) found that $HgCl_2$ exposed to a mercury vapor lamp became cloudy due to the formation of $HgCl$. Mercury, aluminum, and lead undergo oxidative changes when exposed to radium. The alpha rays seem to be most important in producing this result. In our experiments, alpha rays were completely screened out by the metal of the needles or capsule containing the radium. It is possible that gamma rays produce the opposite effect—that of reduction of the above metals. Dilutions of $HgCl_2$ (1-1000 and 1-50,000) were exposed to $12\frac{1}{2}$ mgm. of radium for 24 hours. The addition of dilute HCl failed to produce a precipitate of $HgCl$, nor was a black residue of mercury and NH_4HgCl formed after placing a few drops of NH_4OH on the paper after filtration. *E. dysenteriae* is able to withstand a 1-200,000 dilution of $HgCl$ and if $HgCl$ were formed it was not present in concentrations sufficient to produce any lethal effects.

It was suggested that secondary radiations from the lead shield surrounding the culture tube may have been instrumental in producing death. In tubes containing no metal salt or salts other than mercury or lead, and enclosed within a lead shield in the same

manner, there was pronounced stimulation in division rate. It was concluded, therefore, that any effect due to secondary radiations from the lead shield was negligible.

According to Russ (1913) secondary radiations are emitted when beta, gamma, or X-rays are absorbed by elements with an atomic weight higher than 40. Protoplasm is made up almost entirely of elements of low atomic weight. Gamma rays produce secondary radiations of the beta type, the hardness of which is directly proportional to the atomic number of the element absorbing the primary radiation. Secondary radiations in the tissues of the animal body are attributable probably to the mineral content of these tissues. In our experiments, the mercury or lead added to the cultures may have been absorbed by the amoebae. These particles of mercury or lead within the cytoplasm of the organisms absorbed gamma rays from the radium, which were re-emitted as beta rays. It seems reasonable that the production of light within an organism would produce some very striking effects. Lead or mercury solutions, irradiated for 12 or 24 hours before inoculating with amoebae, failed to exhibit any killing effect. If this effect is produced, then, by secondary radiations, these radiations cannot be of long duration. It is interesting to note, however, in this connection, that no effect was produced when gold with an atomic weight of 197.2 was used. Mercury, atomic weight 200.6, and lead, atomic weight 207.2, gave distinctive results. Slight differences in atomic weight, then, seem to play an important part in this phenomenon.

If the excited mercury or lead particle, atom, or ion contained within the protoplasm of the amoeba does not release its energy in the form of light, it may transfer this energy to another particle or molecule with which it collides. This is termed radiationless transfer or collision of the second type. If this energy is transferred to an essential component of the protoplasm, the effect produced may be sufficient to cause death of the organism. It is not absolutely necessary to assume that the mercury or lead is absorbed. The above energy changes may take place through the surrounding medium, but it is reasonable that they would be much more effective if occurring within the cytoplasm.

The removal of essential bacterial flora would not be responsible for the lethal effect after a 12-hour irradiation. It is possible, however, that the dying out of cultures after two or three transplants

may be due to the absence of certain bacteria which were affected by the action of the metal and radium.

It is obvious that the effect of radium in combination with heavy metal salts is of an entirely different order than the action of radium alone. Very few, if any, morphological changes were noted in amoebae surviving this treatment.

The above is merely a hypothesis set forth in an attempt to explain the phenomena observed in our experiments. It is not within the scope of this work to venture anything further than this.

Several factors may be responsible for the opposite results obtained when the radium plaque was used. In the first place the radium is spread out over a large surface and is not concentrated in a point to the degree that it is in the needles. It is impossible to apply the plaque in such intimate contact with the amoebae as can be done with the needles. The alpha and beta rays may have played some part in overcoming the effects of the gamma rays and metal salt. The use of aluminum as a screen may have been unfortunate as the very soft type of secondary radiation emitted by the screen may have been sufficient to alter the results.

SUMMARY AND CONCLUSIONS

1. The division rate of *Endamoeba dysenteriae* *in vitro* is stimulated from two to four times over that of the controls by exposure to radium radiations.
2. The stimulation continues for not more than 24 hours after the removal of the radium and is followed by a decided retardation of the division rate.
3. Screening out the beta rays with aluminum does not remove the stimulative effect although stimulation may not be so marked as when unscreened radiations are employed.
4. The after effect of radiation on retardation in division rate is not so prominent when the beta rays are removed.
5. Radiated cultures bear transplanting indefinitely. If death did occur it might be ascribed either to the direct effect of the radiations on the amoebae, or to the modifications induced by the radiations on certain bacteria related to the growth of the amoebae.
6. Radium produces morphological changes in amoebae in cultures, the most conspicuous being in the structure of the nucleus. Irradiated

amoebae may increase in size, enucleation or autotomy may occur, and the nuclear chromatin may become homogeneous, or disintegrated.

7. A large amount of radium acting for a short time produces more striking morphological changes than a smaller amount acting for a longer time.

8. Gamma radiations are capable of producing characteristic changes in morphology.

9. The effects of radiation persist for four to six days after the removal of the radium and then the culture gradually returns to normal.

10. The remarkable resistance of *Endamoeba dysenteriae* to radium *in vitro* does not suggest the use of this agent in treating cases of human amoebiasis. The response of *E. dysenteriae* *in vivo* to radium may prove to be of a different order from that *in vitro* and it is possible that investigations along this line would give interesting results.

11. *Endamoeba dysenteriae* *in vitro* is killed when exposed to radium in combination with sub-lethal dilutions of mercuric or lead chloride (1-50,000). This effect may be due to the absorption of the metal by the amoebae and the production of secondary radiations, or to radiationless transfer.

The biologist is forced to wait upon the work of the physical chemist before an adequate explanation can be given for this phenomenon.

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EXPLANATION OF PLATES

Figs. 1-14. *Endamoeba dysenteriae*. $\times 2500$. Camera lucida drawings of amoebae from cultures fixed in hot Schaudinn's fluid and stained in iron haematoxylin.

PLATE 16

Fig. 1. Normal amoeba, resting nucleus. Size 12μ .

Fig. 2. Normal amoeba, nucleus in early prophase. Size 12μ by 19μ .

Fig. 3. Amoeba taken from culture exposed to 10-mgm. radium plaque for 24 hours. Small chromatin blob on one side. Size 15μ by 17μ .

Fig. 4. Amoeba without nucleus, from a culture exposed to $37\frac{1}{2}$ mgm. radium in three platinum needles for 12 hours. Size 12μ .

Fig. 5. Amoeba from culture exposed to $12\frac{1}{2}$ mgm. of radium in brass capsule for 12 hours. Peripheral chromatin is pulled away from nuclear wall. Size 13μ by 13.5μ .

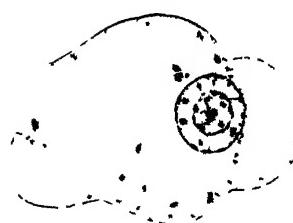
Fig. 6. Amoeba from culture exposed to $37\frac{1}{2}$ mgm. of radium for 12 hours. Peripheral chromatin torn away from nuclear wall and heavily massed on one side. Size 17μ .

Fig. 7. Amoeba from culture exposed to $37\frac{1}{2}$ mgm. of radium for 12 hours. Blobbing of peripheral chromatin on one side. Karyosome faintly stained. Size 21.6μ by 23.5μ .

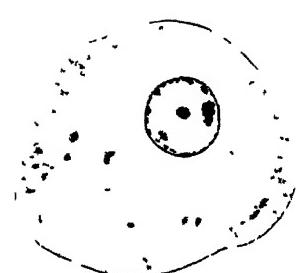
Fig. 8. Amoeba from culture exposed to $37\frac{1}{2}$ mgm. of radium for 12 hours. Peripheral chromatin and karyosome beginning to break up. Size 20μ by 25μ .



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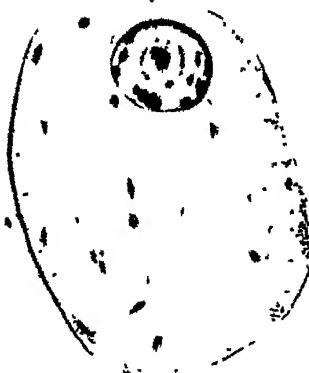
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PLATE 17

Fig. 9. Amoeba from culture exposed to 10-mgm. radium plaque for 72 hours. Peripheral chromatin and karyosome beginning to disintegrate. Size 16μ by 16.5μ .

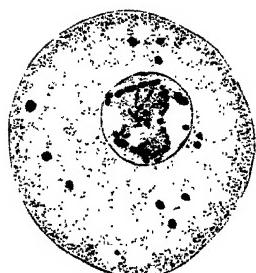
Fig. 10. Amoeba from culture containing 1-50,000 PbCl_2 and exposed to $37\frac{1}{2}$ mgm. of radium for 12 hours. Normal nucleus and several small pseudopods. Blood corpuscle ingested. Size 16μ by 17μ .

Fig. 11. Amoeba from culture containing 1-100,000 HgCl_2 and exposed to $12\frac{1}{2}$ mgm. of radium for 12 hours. Disintegration of chromatin. Size 14.5μ by 22μ .

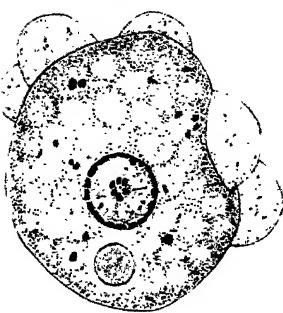
Fig. 12. Amoeba from culture containing 1-50,000 PbCl_2 and exposed to $37\frac{1}{2}$ mgm. of radium for 12 hours. Nucleus in metaphase. Size 15μ .

Fig. 13. Amoeba from culture exposed to $37\frac{1}{2}$ mgm. of radium for 12 hours. Two nuclei, each showing signs of radiation. Size 26μ by 24.5μ .

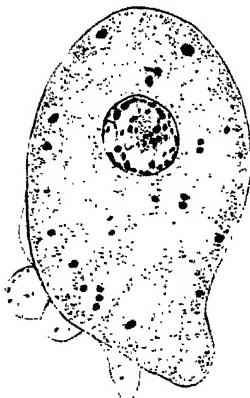
Fig. 14. Amoeba from culture exposed to $37\frac{1}{2}$ mgm. of radium for 12 hours. Nuclear chromatin showing signs of pronounced disintegration. Size 24.4μ by 30.8μ .



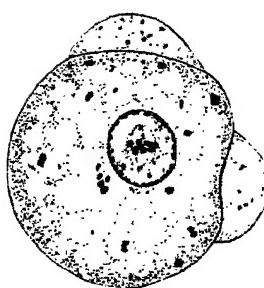
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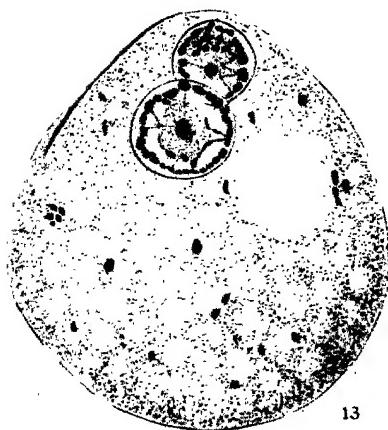
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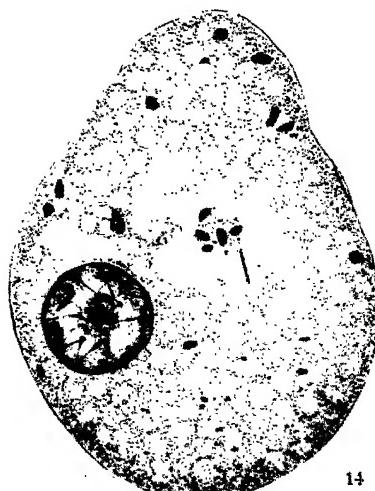
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SNYDERELLA AND CORONYMPHA, TWO
NEW GENERA OF MULTINUCLEATE
FLAGELLATES FROM TERMITES

BY

HAROLD KIRBY, JR.

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY
Volume 31, No. 18, pp. 417-432, plates 18, 19, 2 figures in text
Issued January 29, 1929

UNIVERSITY OF CALIFORNIA PRESS
BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS
LONDON, ENGLAND

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INTRODUCTION

In this paper will be described two new genera of multinucleate flagellates, each of which presents characters of much interest in connection with the study of evolutionary development in the family Calonymphidae of the order Polymastigida.

The family Calonymphidae is composed of xylophagous, multinucleate flagellates which live in termites of the family Kalotermitidae and there, so far as is known, only in the subfamilies Kalotermitinae and Hodotermitinae. In these flagellates the unit of structure is a mastigont, or single neuromotor system. This consists of an axial filament, a parabasal body, one or more blepharoplasts, and several flagella which, in those species that have been carefully investigated for this point, number four. In the more primitive members of the family each mastigont is associated with a nucleus and therefore is termed a karyomastigont. The genus *Stephanonympha*, in which the karyomastigonts number in different species from forty or less to several hundred, falls into this category. Bernstein (1928) described as *Stephanonympha dogieli* a flagellate from *Hodotermes murgabicus* from Turkestan. This species has 4 to 20, and occasionally only 1 to 3 karyomastigonts, arranged irregularly in the anterior portion of the body. It differs from the described species of *Stephanonympha* in

the number and distribution of nuclei and in the structure of the axostyles, to a degree which is probably sufficient to justify its assignment to a different genus.

Diplonympha in many respects resembles *Stephanonympha*, but, according to Grassi (1917), each karyomastigont in it possesses two or sometimes three nuclei arranged in a column vertical to the surface of the body. In *Calonympha* the anterior mastigonts are without nuclei and consequently are termed akaryomastigonts. These are greatly in excess of the karyomastigonts, which constitute the posterior group of neuromotor units. In these genera the nuclei are located in the peripheral cytoplasm in close contact with the blepharoplasts, and the axostyles are gathered into a stout bundle which often projects beyond the posterior limits of the ovoidal cytosome.

Calonymphid flagellates have been found in twenty-two out of twenty-six Kalotermiteinae which the writer has investigated for Protozoa. In *Kalotermes (Lobitermes) longicollis* a new genus has been discovered which represents the most advanced development of the Calonymphidae known at present. The writer is glad of the opportunity to name this genus *Snyderella* for Dr. T. E. Snyder, of the United States Department of Agriculture, in recognition of his contributions to our knowledge of the termites of Central America and elsewhere and of his assistance to the writer in the study of the termite hosts of Protozoa. For the other genus here described, which is more primitive in structure than other known Calonymphidae, *Coronympha* is an appropriate name because of its crown-like circle of sixteen nuclei.

Although both species were observed while living, the descriptions have been made for the most part from smears fixed in Schaudinn's or in a few cases Flemming's fluid without acetic acid and stained in Heidenhain's or Delafield's haematoxylin with or without counter-stain. Delafield's haematoxylin serves especially well for demonstration of the parabasal bodies.

Four species of termites were found to contain these flagellates. Two of them were obtained in 1925 in Panama, *Kalotermes clevelandi* by Mr. J. Zetek and *Kalotermes (Lobitermes) longicollis* by the writer. *Kalotermes immigrans* was collected in 1924 at Fanning Island while the writer was a member of an expedition from the University of California supported by the owners, Fanning Island, Ltd. An undetermined species of *Kalotermes* was brought from Gardner Island in the Galapagos Archipelago by Mr. William Beebe

in 1925 and sent to the writer by Dr. A. E. Emerson. The Central American collections were made with the aid of a grant from the Bache Fund of the National Academy of Sciences, given to Dr. L. R. Cleveland. Part of this work was done while the writer was a member of the staff of the Osborn Zoological Laboratory, Yale University. In addition to those mentioned above, acknowledgment is due to Dr. T. E. Snyder for identification of the termites, to Dr. C. W. Stiles for assistance in nomenclature, and to Miss Lisbeth Krause, artist for the Osborn Zoological Laboratory, for careful preparation of some of the illustrations on the plates.

Snyderella tabogae gen. nov., sp. nov.

Plate 18; figure A

Snyderella tabogae is the largest of seven species of flagellates which occur in the intestine of *Kalotermes (Lobitermes) longicollis* from Taboga Island, Panama. Despite the abundance of Calonymphidae in Central American Kalotermitinae, only this one of fourteen species collected contained *Snyderella*, which is the most advanced of the polymastigote flagellates in development. Associated with this flagellate in its host occurred *Stephanonympha*, *Devescovina*, *Oxymonas*, a trichomonad, and two species of small flagellates.

Snyderella tabogae is larger than most of its relatives and has the ovoidal form possessed by other Calonymphidae, but the anterior end is often especially narrow, conical, and prolonged (fig. A; pl. 18, figs. 11, 12). Twenty individuals from a number of slides ranged in length from 77 to 150 microns, and in breadth from 53 to 97 microns, while an unusually large specimen was 165 and another 172 microns long. The average of these figures, 109 \times 73 microns, is probably less than the actual average size, for many of the largest individuals were broken on the smears and could not be measured satisfactorily.

Of the akaryomastigonts there are at least many hundred and in some cases certainly between two and three thousand. Each (pl. 18, figs. 3-5) consists of an axostyle, a parabasal body, four blepharoplasts, and four flagella. These neuromotor units are distributed through a large proportion of the peripheral region of the body (pl. 18, fig. 12) so that the flagella arise over most of the surface excepting a posterior region which amounts to not more than a quarter of the total length. In some cases (fig. A) the sides of the body are occupied entirely, leaving only the most posterior portion free. At the anterior

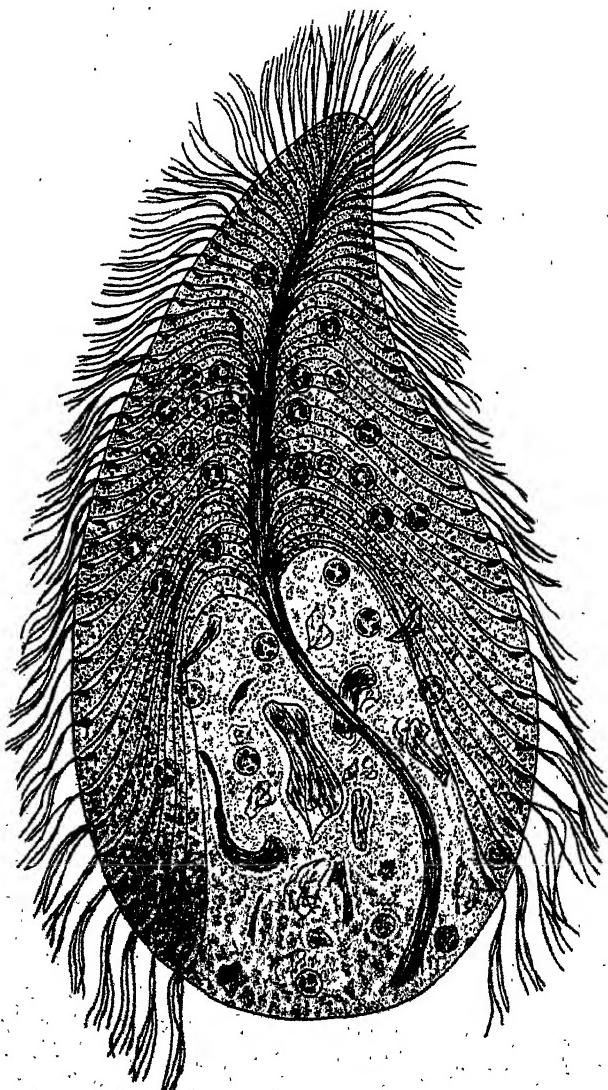


Fig. A. Diagram of *Snyderella tabogae* gen. nov., sp. nov. $\times 700$. The dimensions are those of an individual of large size (172 microns long, while the average is 109). The mastigonts are shown in only one plane, and not all the nuclei are included in the figure. Diagrammed in each mastigont are the four flagella, the parabasal body, the axostyle with its expanded head or capitulum, and the blepharoplasts (the four not distinguishable). Particles of wood are in the cytoplasm.

end, where the cytosome narrows to a conical, rounded tip, the mastigonts are smaller and closer together than they are elsewhere.

In the longitudinal axis of the body is a stout bundle composed of the posterior portions of axostyles which anteriorly turn off toward the periphery. Except near the tip of the body, the axostyles join the central bundle at a point anterior to the blepharoplasts and flagella with which they are associated, so that those of the most posterior mastigonts are much reflexed. The axial bundle does not preserve a straight course, and the filaments, which do not continue as far as the posterior limits of the body, often spread apart near its end.

Near the blepharoplasts each axostyle, which even in its narrowest portion has a double contour, is enlarged and flattened into a capitulum (pl. 18, figs. 1, 2) in a plane coincident with that of a radial section. Directly beneath the more convex anterior margin of this capitulum, against which the parabasal body lies, is a row of granules which are probably homologous with those which occur within the axostyles of many trichomonad flagellates.

Situated a short distance beneath the pellicle at the end of each axostyle are four small blepharoplasts (pl. 18, fig. 5) from each of which a flagellum arises. Three of the blepharoplasts, and with them the flagella, are grouped separately from the fourth. The flagella are relatively rather short (fig. A), those of the more anterior mastigonts measuring about 20 microns and the more posterior about 25 microns. This condition is in marked contrast to that of other known Calonymphidae in which the flagella are generally as long as or longer than the body of the flagellate.

In material stained in Delafield's haematoxylin of suitable intensity rather large granules appear; located just beneath the periplast and distributed closely throughout the portion of the body from which flagella arise (pl. 18, fig. 11). These are the parabasal bodies, the presence of which cannot easily be recognized in material stained only with Heidenhain's haematoxylin. They range in size and form from minute granules in the anterior mastigonts to elongated, slightly curved bodies measuring 2 to 3 microns in length in the more posterior neuromotor units (pl. 18, figs. 3, 4, 8-10).

The nuclei are in the endoplasm, together with numerous axostyles, particles of wood, and many small granules. Fragments of wood are absent from the conical anterior portion of the body where the axostyles are especially numerous, but are abundant elsewhere. Around

the posterior part of the cytosome, especially where there are no mastigonts, is a zone of finely granular cytoplasm which is free from coarse granules such as those which occur in the endoplasm.

The distribution of nuclei is suggestive of that in *Opalina* and indeed the nuclei of the two organisms are somewhat similar morphologically. In *Stephanonympha* and *Calonympha* the nuclei are located close to the surface of the body and each nucleus is associated with a mastigont. Neither of these facts is true of *Snyderella* in which the nuclei are distributed without regularity in the "deeper endoplasm. No connections between any of the plepharoplasts and the nuclei have been found (fig. A; pl. 18, fig. 12). In the most anterior portion of the body, where the axial filaments are crowded and the larger cytoplasmic inclusions are absent, nuclei are generally not present. Exceptionally a few nuclei are located in this anterior region beneath the layer of parabasal bodies and between the axostyles, which are "spread apart to accommodate them. Among the more posterior axostyles and in the wodd-containing part of the cytoplasm, nuclei are more numerous.

The number of nuclei is variable in different individuals. In twenty cases this number ranged from 28 to 65, averaging 45, and of these twenty only one had the same number as another. Even and odd numbers occurred with equal frequency. There were probably some with a greater number of nuclei than 65, for many of the largest individuals had been ruptured and some of the nuclei discharged from the body.

The nuclei (pl. 18, figs. 6, 7), which are spherical, vary in diameter from 3 to 5 microns, 4.5 being the most frequent size. The space within the membrane seems to be filled with a finely alveolar protoplasm, or possibly an achromatic reticulum. Except for this protoplasm and in a few cases a small number of granules, the central portion of the nucleus contains no structures. In its outer portion are several large chrcmotic masses and numerous small chromatic granules. Of the larger masses, which vary greatly in form and size, there are in different nuclei from two to nine, although the more usual number is from four to six. Two of them are sometimes connected by a stout chromatic strand. In various nuclei, a transitional series may be found between the smallest granule and the largest mass, but for the most part the smaller granules, which are abundant in the peripheral zone, form a distinct structural entity. No two nuclei present exactly the same appearance in internal structure.

Spirochaetes are almost always present in large numbers, each attached by one end to that part of the surface which is free of flagella (pl. 18, fig. 11). Similar microorganisms are frequently present on *Calonympha*, *Stephanonympha*, and other Calonymphidae, as well as on many other flagellates of termites.

The genus *Snyderella* is advanced beyond *Calonympha* in the greater number of akaryomastigonts, greater extent of the area through which they are distributed, larger size of body, shorter flagella, and distribution of nuclei in the deeper endoplasm at a distance from the mastigonts. There is a suggestion of the hypermastigote condition in the large number of flagella which corresponds to each nucleus. The Hypermastigida, however, have developed in a very different direction from the Polymastigida. In the former the uninucleate condition is retained and the number of flagella increases, while in the latter, in the Calonymphidae, a multinucleate condition develops. In the more primitive genera each nucleus is associated with four flagella and other structures of a karyomastigont, while in others akaryomastigonts have developed in excess of the number of nuclei. In *Snyderella* the nuclei have migrated into the deeper portions of the body where they are distributed without regularity and seem to have no connection with the blepharoplasts during the period of vegetative activity.

GENERIC DIAGNOSIS

Snyderella gen. nov.

Multinucleate flagellates with a variable number of nuclei irregularly distributed in the deeper cytoplasm, at a distance from the peripheral neuromotor structures. The unit of structure is a mastigont, or single neuromotor system, with four flagella, blepharoplasts, a parabasal body, and an axostyle. The numerous mastigonts are close together and extend through the greater part of the peripheral region of the cytoplasm. The axostyles are collected into a bundle posterior to the middle of the body.

Type species.—*S. tabogae* from *Kalotermes (Lobitermes) longicollis* (Banks, 1918) from Taboga Island, Panama.

Type specimens deposited in United States National Museum and the Department of Zoology at the University of California.

Coronympha clevelandi gen. nov., sp. nov.

Plate 19; fig. B

Among the termites of the subfamily Kalotermitinae obtained in Central America, one species differed markedly from the others in its flagellate fauna in that it contained a unique, undescribed genus of multinucleate flagellate. The host was at first determined as *Kalotermes marginipennis*, but when the protozoan fauna was found to differ greatly from that of other termites given the same name, the systematic characters were reinvestigated by Dr. Snyder and justification found for the description of a new species, *Kalotermes clevelandi* Snyder. The flagellate, which will be described as *Coronympha*, had previously been seen by the writer in *Kalotermes immigrans* Snyder from Fanning Island, and later was found in an undescribed species of *Kalotermes* from Gardner Island in the Galapagos Archipelago. As there appear to be no important differences between the flagellates of this genus in the three hosts, all will be included in the species *C. clevelandi* of which the principal part of the description has been prepared from the infection of *Kalotermes clevelandi*.

In contrast to the large, diversified faunas of many termites of the genus *Kalotermes*, the three species containing *Coronympha* have few species of Protozoa. In each case *Coronympha* dominates the fauna in abundance. In *K. clevelandi* and the Galapagos species there occurs also a species of *Trichonympha* and a very small flagellate, but these are lacking on the slides made from *K. immigrans*. Each termite contains in addition a uninucleate oxymonad, which is especially large and abundant in some material from the Galapagos species. The fauna of *Kalotermes marginipennis* is very different, consisting of eight or nine species including *Devescovina*, *Calonympha*, an undescribed calonymphid flagellate, *Trichonympha*, *Oxymonas*, and some small forms.

Coronympha clevelandi is ovoidal (fig. B, 1; pl. 19, fig. 19), often nearly as broad as long, and frequently has a shallow, rounded protuberance at the anterior end. Fifty individuals from *K. clevelandi* averaged 30×23 microns, ranging from 25×18 to 53×46 microns. In the flagellates from the other two hosts the average size is somewhat less. The forty-eight long flagella, which arise in a single anterior circle (pl. 19, fig. 18; fig. B, 3) are prominent external structures. Otherwise the body surface is naked, as there are no microorganisms like those which adhere to the bodies of many flagellates of termites.

There are sixteen karyomastigonts arranged in a single circle (pl. 19, fig. 18), each (fig. B, 2) consisting of four flagella, a blepharoplast, a chromatic rod, rhizoplast, axostyle, parabasal body, and nucleus. The sixteen blepharoplasts form a small circle close to the

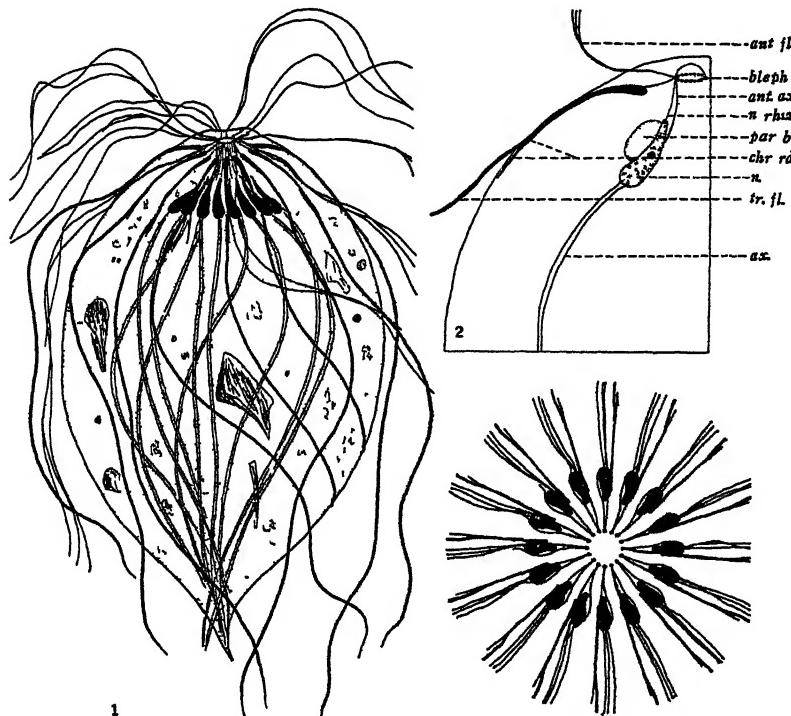


Fig. B. Diagrams of *Coronympha clevelandi* gen. nov., sp. nov. 1. Based on average size. The nuclei are shown uniformly black as they appear in material stained so as to demonstrate also flagella and axostyles. $\times 2000$. 2. Diagram of a single karyomastigont. The entire ring of blepharoplasts is shown. $\times 4000$. 3. Apical view of karyomastigonts. $\times 1830$.

Abbreviations: *ant. ax.*, anterior portion of axostyle; *ant. fl.*, anterior flagella; *ax.*, axostyle; *bleph.*, blepharoplasts; *chr. rd.*, chromatic rod; *n.*, nucleus; *n. rhiz.*, nuclear rhizoplasts; *par. b.*, parabasal body; *tr. fl.*, trailing flagellum.

tip of the body. Above this circle (pl. 19, fig. 14) and beneath the outer border of the cytoplasm is a more deeply staining, rounded, cap-like area. The chromatic rods (fig. B, 2, 3), which are rounded proximally and taper to fine filaments distally, lie in the peripheral cytoplasm where they radiate from an imaginary circle the diameter of which is about three times that of the circle of blepharoplasts. Three of the flagella are fine, while the fourth is stouter than this group, and all are somewhat longer than the body. The stout flagellum is closely associated at its base with the chromatic rod (pl. 19, fig. 17),

so that in many cases the two cannot be distinguished separately. The flagella and chromatic rods are comparable to similarly differentiated structures in devescovinids, and indicate the possible existence of some relationships between these flagellates and *Coronympha*.

Those portions of the axostyles which are between the blepharoplasts and the nuclei form a cylindrical figure (fig. B, 1; pl. 19, fig. 14). Each axostyle approaches the apex of the nucleus, then turns (fig. B, 3) and continues along its right side, broadening from a filament to a rod-like structure (fig. B, 2, *ax.*). Back of the nucleus it passes through the endoplasm independently of the other axostyles (pl. 19, fig. 19), which do not form a central bundle as in *Stephanonympha*, *Calonympha*, and *Snyderella*. At the posterior end of the body, however, these structures often meet and form a caudal projection (fig. B, 1) which is sometimes fairly long. In a few cases there are two such projections, some axostyles protruding independently of the principal group.

Each nucleus is club-shaped, with the smaller end anterior, and slightly curved, with the concave side outward (fig. B, 2). From the posterior end of the cylinder constituted by the anterior portions of the axostyles, the sixteen nuclei diverge, so that the figure formed is that of an inverted funnel with short, fairly stout stem (pl. 19, fig. 14). As this is seen from the anterior end (pl. 19, fig. 18), the nuclei radiate like the petals of a flower from the inner circle of blepharoplasts. The diameter of the circle touched by the outer ends of the nuclei is, in larger individuals, about twelve microns. There is, however, much variation in this measurement, as there is in the size of the nuclei in individuals of different sizes. Within the nuclear membrane (pl. 19, fig. 15; fig. B, 2, *n*) there are one or more relatively large, deeply staining granules, the endosomes, which are variable in size, number, and position. Besides these bodies, finely divided chromatin fills the nucleus. There appears to be a fine rhizoplast (fig. B, 2, *rhiz.*) connecting the apex of the nucleus with the corresponding blepharoplast.

The spherical or ovoidal parabasal bodies (fig. B, 2, *par. b.*), which are fitted into the outer concavities of the nuclei, have a maximum length exceeding half that of the nuclei (pl. 19, fig. 14). In many cases, however, especially in smaller individuals, they are proportionately smaller than this. The flagellates from *Kalotermes immigrans* (pl. 19, figs. 13, 16) and the Galapagos termite have parabasal bodies which are relatively smaller than those of the species from

K. clevelandi, but there is so much variation in the size of this structure that one is not justified in using its measurements as a specific character.

In some individuals, the granular cytoplasm is free from foreign inclusions, but the majority contain small particles of wood or, less frequently, fragments large enough to distort the cytosome. There are also many small granules, larger, deeply staining globules, and, not infrequently, a parasite similar to *Sphaerita*.

Coronympha clevelandi, which is simpler in structure than other known species of Calonymphidae, bears some resemblance to the devescovinid flagellates. Each mastigont of the multinucleate form may be compared with the uninucleate flagellate. In the devescovinids and calonymphids there are four flagella, of which one is separate from and frequently stouter than the others. This differentiation is conspicuous in the devescovinids and *Coronympha*, but it is less evident in higher members of the Calonymphidae. *Coronympha*, as well as the devescovinids, possesses a chromatic rod associated with the base of the trailing flagellum. In the structures of this flagellate, then, there are some evidences of the divergence of Devescovinidae and Calonymphidae from a common ancestor. This probability also gains support from a consideration of their distribution: these two families of polymastigotes have been found exclusively in the Hodotermitinae and Kalotermitinae.

GENERIC DIAGNOSIS

Coronympha gen. nov.

Multinucleate flagellates, each with sixteen nuclei in a single circle in the anterior portion of the body. Each nucleus is associated with a blepharoplast, nuclear rhizoplast, chromatic rod, three long fine anterior flagella, a long stout trailing flagellum, an axostyle, and a parabasal body. The sixteen blepharoplasts form a small circle at the apex of the body, and from it radiate the chromatic rods, the trailing flagella, and the anterior flagella. The club-shaped nuclei contain one or more endosomal masses and finely distributed chromatin. Close to each nucleus is a parabasal body, and backward from each nucleus runs a fairly stout axostyle, which connects anteriorly with the corresponding blepharoplast.

Type species.—*C. clevelandi* from *Kalotermes clevelandi* Snyder, 1926, from Ancon, Canal Zone, Panama.

Type specimens deposited in United States National Museum and the Department of Zoology at the University of California.

Additional hosts.—*Kalotermes immigrans* Snyder, 1922, from Fanning Island; *Kalotermes* sp. from Gardner Island, Galapagos.

SUMMARY

1. *Snyderella tabogae* from the termite *Kalotermes (Lobitermes) longicollis* Banks, 1918, is described and figured as the type species of a new genus.
2. This flagellate is rounded posteriorly, bluntly conical anteriorly. The average size is about 109×73 microns.
3. There are 28 to 65 or more nuclei, averaging about 45. They are in the deeper endoplasm and not directly associated with mastigonts. These nuclei are spherical, about 4.5 microns in diameter, and contain 4 to 6 or more peripheral chromatic blocks in addition to numerous small chromatic granules.
4. Akaryomastigonts, each of which consists of four flagella, four blepharoplasts, an axostyle, and a parabasal body, extend through a large proportion of the peripheral zone of the body.
5. The flagella are from 20 to 25 microns long. Spiral organisms adhere to the posterior part of the body surface.
6. The axostyles are broadened and flattened at the outer end, and are collected into a bundle near the middle of the body.
7. *Snyderella* is the most advanced genus of the Calonymphidae known at present, having more non-nucleate mastigonts than others while the nuclei are no longer in close association with mastigonts nor limited in distribution to the peripheral region of the cytoplasm.
8. *Coronympha clevelandi*, from *Kalotermes clevelandi* Snyder, 1926, *Kalotermes immigrans* Snyder, 1922, and *Kalotermes* sp. from the Galapagos Islands, is described and figured as the type species of a new genus.
9. *Coronympha clevelandi* is ovoidal in form and averages about 30×23 microns in size.
10. There are sixteen nuclei in a circle near the anterior end of the body. Each nucleus is club-shaped and slightly curved, and contains one or more endosomes and finely divided chromatin.
11. With each nucleus is associated a mastigont composed of four flagella, a chromatic basal rod, a blepharoplast, an axostyle, a parabasal body, and a rhizoplast connecting the blepharoplast to the nucleus.
12. The flagella are longer than the body and are differentiated into three fine anterior flagella and an unusually stout trailing flagellum.

13. The chromatic rod is closely associated with the trailing flagellum at its base. This is a new structure in the Calonymphidae.

14. The sixteen blepharoplasts lie in a circle close to the tip of the body.

15. The axostyles arise from the blepharoplasts and continue past the nuclei to the posterior end of the body, beyond which they sometimes project. They are not collected into a bundle as in other known Calonymphidae.

16. Both *Snyderella tabogae* and *Coronympha clevelandi* ingest fragments of wood. The latter is often parasitized by an organism similar to *Sphaerita*.

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EXPLANATION OF PLATES

PLATE 18

All figures are of *Snyderella tabogae* gen. nov., sp. nov. from *Kalotermes (Lobitermes) longicollis* Banks, drawn with camera lucida except figs. 3 and 4. Figures 11 and 12 drawn by Miss Lisbeth Krause. Abbreviations for methods of preparation: S., Schaudinn's fluid; D., Delafield's haematoxylin; H., Heidenhain's haematoxylin; F., acid fuchsin.

Fig. 1. Expanded outer portion of axostyle, 3-1 grouping of flagella and parabasal body. $\times 1500$.

Fig. 2. Similar, showing origin of flagella and granules within axostyle. S.H.F. $\times 1500$.

Fig. 3. Diagrammatic figure of mastigont, viewed from side, showing arrangement of flagella, expanded head of axostyle, granules in axostyle, and parabasal body.

Fig. 4. Same, viewed from posterior edge, with parabasal above.

Fig. 5. End view of mastigont, showing origin of flagella and four blepharoplasts. S.H.F. $\times 1500$.

Figs. 6-7. Nuclei. S.H.F. $\times 1500$.

Fig. 8. Portion of edge of body, showing parabasals. S.D. $\times 1500$.

Figs. 9-10. Surface views of portions of ruptured bodies, showing distribution of parabasal bodies. S.D. $\times 1500$.

Fig. 11. Surface view, showing distribution of parabasal bodies, flagella, and adherent spirochaetes. S.D. $\times 426$.

Fig. 12. Optical section of individual drawn also in fig. 11, showing mastigonts, axostyles, nuclei, and fragments of wood in cytoplasm. S.D. $\times 426$.



11



12



PLATE 19

All figures are of *Coronympha clevelandi* gen. nov., sp. nov., drawn with camera lucida. Figures 18 and 19 drawn by Miss Lisbeth Krause. From *Kalotermes clevelandi* unless stated otherwise.

Fig. 13. Nuclei and parabasal bodies of a very small individual from *K. immigrans*. S.D. $\times 2036$.

Fig. 14. Semischematic drawing showing nuclei, parabasal bodies, and other neuromotor structures of a large individual from *K. clevelandi*. S.H.F. $\times 2036$.

Fig. 15. Nuclei, showing chromatin and endosomes. S.H. $\times 2036$

Fig. 16. Nuclei and parabasal bodies of *Coronympha* from *K. immigrans*. S.D. $\times 2036$.

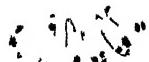
Fig. 17. Semischematic apical view of mastigonts. The chromatic rods can in some cases be distinguished from the stout flagella. S.H.F. $\times 1500$.

Fig. 18. Apical view, showing circles of blepharoplasts and nuclei, and origin of flagella. S.H.F. $\times 1500$.

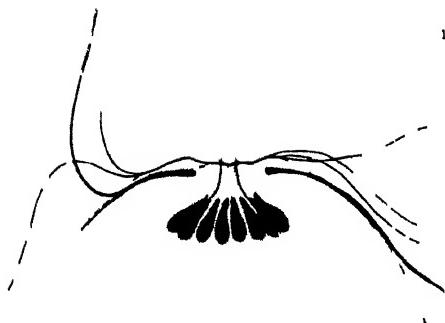
Fig. 19. Lateral view showing clavate nuclei, axostyles, and flagella. S.H.F. $\times 1500$



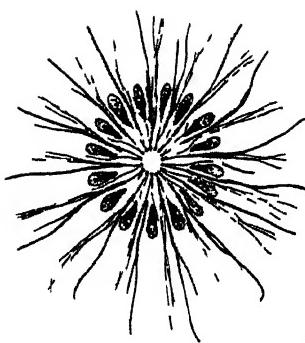
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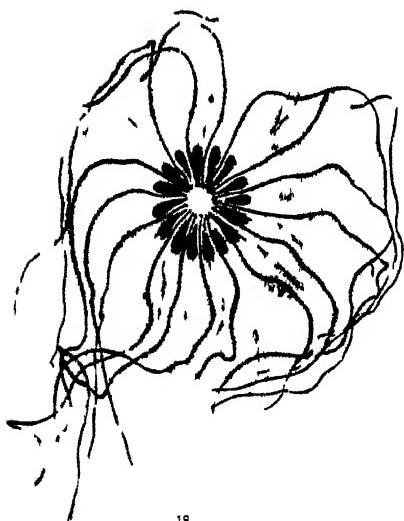
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NATURAL AND ARTIFICIAL PRODUCTION OF
SO-CALLED "MITOTIC FLARES" IN THE
INTESTINAL FLAGELLATES OF
TERMOPSIS ANGUSTICOLLIS

BY

BESSIE J ANDREW AND S F LIGHT

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY

Volume 31, No. 19, pp. 433-440

Issued January 29, 1929

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

NATURAL AND ARTIFICIAL PRODUCTION OF SO-CALLED "MITOTIC FLARES" IN THE INTESTINAL FLAGELLATES OF *TERMOPSISIS ANGUSTICOLLIS*

BY

BESSIE J. ANDREW AND S. F. LIGHT

The majority of workers making studies of the morphology and cytology of the Protozoa of various termites have reported a remarkable paucity or entire lack of mitotic figures in the greater number of their stained slides, while a rare smear will be very rich in individuals of one or several species in one or all of the stages of mitosis. This fact, in connection with the tacitly accepted theory stated by Cleveland (1925a) that the death rate of the Protozoa is enormous, led naturally to the hypothesis that epidemics of divisions occurred periodically, brought on by unknown factors, possibly by external conditions such as temperature and moisture, or by some peculiar internal physicochemical change within the termite, perhaps a hormone production, or due to a natural rhythmic reproduction cycle within the Protozoa themselves.

The possibility of inducing mitotic figures at will in any form is of considerable interest, and this is particularly true of such organisms as are found in enormous numbers in the termite intestine, with their great complexity and their peculiar ecological relationships. The present work was begun with the hope of ascertaining the reason for the phenomenon known as "mitotic flare" and of finding some means of stimulating division.

The termite used for the investigation was *Termopsisis angusticollis* Hagen, which, in contrast to most termites, is a splendid laboratory animal and is to be had in large colonies. The colonies used were from the San Francisco Bay region of California. The larger Protozoa of this species and their mitotic phenomena are well known from the work of Kofoed and Swezy (1919: I, II, III, IV).

The random observations of one of us (S. F. L.) had indicated what was noted early in the investigation, that there seemed to be few

fragments of dead organisms within the intestines, a surprising observation in view of the accepted theory of a high death rate. This opened up three possibilities: (1) the Protozoa upon dying disintegrate immediately and completely; (2) after death they retain their normal appearance for some time and then disintegrate very rapidly; or (3) contrary to belief, the number dying is comparatively small.

The first two possibilities will be discussed in some detail in a paper soon to appear on other problems. For the present paper a consideration of results obtained by a simple staining method will be sufficient. It was found that a saturated solution of water-soluble eosin in normal salt, much diluted with tap water gave an excellent diagnostic stain. Most of the intestinal débris and any dead Protozoa quickly absorbed the characteristic eosin red or pink, while living animals appeared pale green, the complementary color, apparently a case of simultaneous color contrast. By this method it is easy to determine the relative numbers of dead and living animals and to detect the presence of partly decomposed forms. Studies were made by this method and others of the intestinal contents of termites (1) under normal conditions taken at all times of the day and night, (2) during starvation, (3) after dying, (4) after partial and (5) after complete defaunation by oxygenation and pressure, (6) after treatment with carbon dioxide, and (7) of intestinal contents left for varying periods in different media before addition of the eosin; by these methods it was always found possible to detect disintegrating forms when present, even in partly defaunated animals where their proportion was high.

In all, more than three thousand examinations of intestinal contents were made, using one or the other of (1) unstained living material, or (2) living material stained with dilute eosin or eosin-iodine or methylene blue, or (3) smears stained in Heidenhain's haematoxylin. These numerous examinations have revealed so minute a percentage of decomposing Protozoa in the normal intestine that there seems to be no basis for assuming an even moderately high death rate among them.

This explains in a simple manner the absence, or isolated character, of mitotic figures in most of the smears made by investigators studying the Protozoa. Under normal conditions, once the optimum number is reached, the fauna will remain fairly constant with but little change as to its individual make-up. If, then, under normal conditions the death rate is low, how can we explain the occasional wealth of mitoses?

Studies were made of various methods for reducing the fauna, such as (1) heating and (2) starvation as described by Cleveland (1924, 1925a), (3) reduction of moisture by exposure to the atmosphere, (4) defaunation by oxygenation and pressure as discovered by Cleveland (1925b) and as developed by Light and Sanford (1928), (5) by treatment with carbon dioxide, and (6) by pressing out intestinal contents. The first three would be so infrequently encountered in the successful colony in nature as to render them improbable explanations of the commonly encountered "mitotic flare" and the last three are, of course, totally unnatural. Attention was finally focused, therefore, on ecdysis.

This process in the termites *Termopsis angusticollis* and *T. nevadensis* has been admirably treated by Heath (1927) and Heath and Wilbur (1927), who present the concisely summarized findings of years of investigation and observation on the occurrence, duration, and changes within the various instars of the different castes of the genus, with their possible significance in relation to caste formation. Mention of particular instars below is based on identifications made from their descriptions. As space forbids extensive quotations from their papers, reference should be made to them for the distinguishing features of these stages in the termites.

The marked changes that have been found in the fauna during this time will be more fully considered in a later paper. Germane to this investigation is the fact that by the close of the molting process the xylophagous Protozoa, i.e., *Trichonympha campanula* Kofoid and Swezy, *Leidyopsis sphaerica* Kofoid and Swezy, and *Trichomonas termidis* Kofoid and Swezy, have so entirely disappeared from the intestine, that termites isolated from the group at this time fail to develop these elements of the normal fauna, and the non-xylophagous form has also either disappeared or become greatly reduced in numbers.

It appeared, therefore, that the "mitotic flares" under consideration might be due, not to a rhythmic cycle characteristic of the Protozoa or their metabolism, but to the refaunations following successive moultings of the termites. The cycle thus conceived would consist, therefore, as regards the termite, of (1) A brief defaunate period immediately following ecdysis; (2) a period of normal refaunation soon after ecdysis; following on reinfection due to the normal contacts in the colony; (3) a long period of maximum infestation; (4) a period immediately preceding and including ecdysis during which

defaunation is normally accomplished in some manner not yet clearly made out.

As concerns the Protozoa the cycle might be thought of as consisting of three periods:

(1) A period of rapid multiplication following upon reinfection of the recently moulted termite and ceasing with maximum infestation; (2) a long vegetative period lasting through all save the beginning and end of the particular stadium of the particular termite, during which there is maximum infestation, and the death rate and reproductive rate are very low or practically nil; (3) a short period of reduction of fauna preliminary to and during ecdysis due to elimination from the termite or perhaps to death of the Protozoa.

ARTIFICIAL PRODUCTION OF MITOTIC FLARES

To test this theory the freshly molted animals were taken each day for three weeks during July, 1928, from a mixed laboratory colony numbering between eight and ten thousand individuals and another of about five thousand. Only animals above the third instar (Heath, 1927) were removed, and these numbered from about twenty-five to seventy daily. Whether moltings are as frequent during the winter months has not been determined.

Since ability to recognize recently molted termites would, if the theory proved correct, be of much importance, daily alterations were carefully watched. The color changes are the most noticeable, and, though estimations of the time following the ecdysis by this means are at best empirical ones, they will be found useful. Facility of recognition is best developed by many observations, but by isolating animals of a known molting hour a summary has been made that may be helpful. All these observations were on *Termopsis angusticollis* Hagen from the San Francisco Bay region, California.

First day. The freshly molted nymphs of the reproductive and the soldier castes of the fifth to the ninth instars (Heath, 1927) are almost entirely white, except for the eyes in the late instars of the reproductive caste, and are of a remarkably beautiful translucence. A dark line which may be visible within the abdomen is the old chitinous lining of the intestine not yet discarded. There is a good deal of individual variation in the length of time this appearance lasts, though in general the larger animals become pigmented more rapidly than the

small ones. This is not true of the new soldiers, which may remain with very little pigmentation for several days. In general by the end of twelve hours, the animal will be of a soft cream color, which gradually deepens and becomes warmer by the end of the twenty-four hours. The mouth parts begin to show greater pigmentation during the later part of the first day.

Second day. The process of pigmentation increases as above, but the mouth parts become slightly more red in color and the cream of the body takes on a pinkish tone.

Third day. The animal by this time approximates normal coloration and might readily escape detection by the untrained eye unless looked for. It is generally lighter, however, more pink in color, with lighter and more reddish head and mouth parts.

Fourth day. The termite is somewhat darker and a little more tan, with the head showing browner; there is often little difference from the third day, or it may, on the other hand, look very like the unmolted animals. As by this time refaunation has usually taken place it is probable that the "mitotic flare" smears have ordinarily been made from animals in the third and fourth days after ecdysis.

Fifth to seventh days. Pigmentation increase is very gradual from the fourth day, and determination in unsegregated animals is difficult to make. By the end of a week the return to normal coloration is usually complete except in the new soldiers.

In the earlier instars the only marked color is in the mouth parts, and it is not easy to estimate their changes with time. No differences were noted between termites fed on wood and those on filter paper except as the color of the wood showed through the abdomens. Light exposure seemed to have little to do with pigmentation, since those kept in darkened containers pigmented as rapidly as those in light, although no attempt was made to exclude all illumination.

After about three hundred of these naturally defaunated termites had been accumulated, representing periods of from two days to two weeks after ecdysis, they were placed in Syracuse watch glasses, fifteen to seventeen to each glass, and fed the intestinal contents of normal, faunated animals. This was done by forcing the fluid from the intestine into the dish by a series of slight, rotary pressures on the abdomen of the faunated animal, beginning at the upper end of the abdomen and working downward. *Termopsis* gives up its intestinal contents very readily, and is apparently not injured by the

removal if enough time is left for complete recovery. From three to seven animals were so treated to feed each defaunated group. Feedings of this kind were found better than those in which there was handling of the molted animals.

From termites fed in this way smears were made every three hours from six to thirty hours, and at thirty-six, forty, and forty-eight-hour periods. The fraction faunated was determined by forcing the contents from the intestine as described above and examining under the microscope during the partial drying before fixation. As an added check, intestinal fluid was examined from the same termites two days later, with identical results. About three-fourths of the animals were found to have been refaunated by the one feeding, and ten of the most characteristic smears were kept for each group and stained in Heidenhain's haematoxylin. The result was the appearance of a large percentage of "mitotic flares" in the refaunated termites.

A consideration of the mitotic process in the Protozoa of *Termopsis* is unnecessary here, as the subject has already been very adequately handled by Kofoid and Swezy (1919: I, II, III, IV) and Andrews (1925).

From a study of the series of slides made the following conclusions are drawn:

1. Not all termites become faunated on one feeding of intestinal fluid.
2. The majority of refaunated individuals quickly regain a fauna normal in amount and kind.
3. In so doing they show, over two days and perhaps longer, a very considerable percentage of Protozoa in process of mitosis.

Further, the evidence, while not conclusive, indicates:

1. That there is considerable variation in the rapidity with which division takes place within the intestine of different termites.
2. That the rate of division of the different protozoan species is not the same.
3. That the division within each species approaches but does not reach a geometrical progression.

The usefulness of the method and the problems it opens both for research and class-room use are obvious, particularly as it is so close to the natural one, while still possessing a time control. As a variation where the latter is not needed, termites showing the third and

fourth-day colorings might be used directly from the colony. This will probably be the easiest means of procuring the mitotic stages with other species of termites, if, as is probable, their "mitotic flares" may be explained in the same way, for feeding and injection are more difficult with smaller animals and more delicate Protozoa.

A certain proportion of smears made from one group of *Termopsis* from which most of the intestinal contents had been forced twenty-four hours earlier showed numerous mitotic figures. As this treatment is weakening when large amounts of fluid are removed, better results would probably have been shown if the time had been longer or the amounts taken had been smaller. Mitotic figures could almost certainly be induced by partial starvation, drying, heat, oxygen, or oxygen and pressure if partial defaunations were obtained in each case and the termites then returned to normal conditions, or if complete defauna-tion were followed by feeding or injection of infective material.

SUMMARY

There seems to be no reason for assuming a high death rate among the intestinal flagellates of *Termopsis*, and it seems permissible to infer the same to be true of the faunas of other termites. The low percentage of mitotic figures found by most workers is thus explained. There is also apparently no necessity for supposing that "mitotic flares" occur periodically or occasionally in the normal intestine save as an accompaniment of normal refauna-tion following the molting process, and those reported are to be thus explained. Division figures may best be obtained in the case of *Termopsis* about three days after the casting of the skin in animals left with the colony. With some practice these individuals may be recognized with sufficient accuracy to increase greatly the percentage of smears showing mitotic figures. The flare may be produced artificially in several ways, one of the simplest being the feeding of intestinal contents to the recently molted, isolated termites. Other methods of defauna-tion and refauna-tion are suggested which may be found interesting and useful for certain types of investigations.

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ERRATA

Page 137, line 3 from bottom. *For Bagdanow's read Bogdanow's.*

